SHOUT
SHARE
IT

7857-72

THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. CONKLIN, Princeton University
DONALD P. COSTELLO, University of North Carolina
E. N. HARVEY, Princeton University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University
DOUGLAS WHITAKER, Stanford University

H. B. STEINBACH, University of Minnesota
Managing Editor

VOLUME 99
AUGUST TO DECEMBER, 1950

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between June 15 and September 1, and to Dr. Donald P. Costello, Department of Zoology, University of North Carolina, Chapel Hill, North Carolina, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

LANCASTER PRESS, INC., LANCASTER, PA

CONTENTS

No. 1. August, 1950

PAGE

Annual Report 1

Anderson, John Maxwell

A cytological and cytochemical study of the male accessory reproductive glands in the Japanese beetle, Popillia japonica Newman 49

Grosch, Daniel S.

Starvation studies with the parasitic wasp habrobracon 65

Hand, Cadet and John R. Hendrickson

A two-tentacled, commensal hydroid from California (Limnomedusae, Proboscidactyla) 74

Moore, Anna-Betty Clark

The development of reciprocal androgenetic frog hybrids 88

Salvatore, Carlos Alberto

Action of estrone and progesterone on nuclear volume (studied by applying the Karyometric-statistical method) 112

Spratt, Nelson T., Jr.

Nutritional requirements of the early chick embryo. III. The metabolic basis of morphogenesis and differentiation as revealed by the use of inhibitors 120

Stunkard, Horace W.

Further observations on Cercaria parvicaudata Stunkard and Shaw, 1931 136

No. 2. October, 1950

Bonner, John Tyler

Observations on polarity in the slime mold Dictyostelium discoideum .. 143

Browne, Marie J., Marjorie W. Pitts, and Robert F. Pitts

Alkaline phosphatase activity in kidneys of Glomerular and Aglomerular marine Teleosts 152

Frenkel, Albert, Hans Gaffron, and E. Battley

Photosynthesis and photoreduction in blue-green algae 157

Wells, Patrick H., and Arthur C. Giese

Photoreactivation of ultraviolet light injury in gametes of the sea urchin Strongylocentrotus purpuratus 163

Herlant-Meewis, Henriette

Cyst-formation in Aeolosoma hemprichi (Ehr) 173

Moore, Hilary B.

The relation between the scattering layer and the Euphausiacea 181

65213

PEISS, C. N., AND JOHN FIELD
The respiratory metabolism of excised tissues of warm- and cold-adapted fishes . . . 213
SCHOLANDER, P. F., VLADIMIR WALTERS, RAYMOND HOCK, AND LAURENCE IRVING
Body insulation of some arctic and tropical mammals and birds . . . 225
SCHOLANDER, P. F., RAYMOND HOCK, VLADIMIR WALTERS, FRED JOHNSON, AND LAURENCE IRVING
Heat regulation in some arctic and tropical mammals and birds . . . 237
SCHOLANDER, P. F., RAYMOND HOCK, VLADIMIR WALTERS, AND LAURENCE IRVING
Adaptation to cold in arctic and tropical mammals and birds in relation to body temperature, insulation and basal metabolism . . . 259
TAYLOR, WILLIAM RANDOLPH
Reproduction of Dudresnaya crassa Howe . . . 272
TURNER, C. L.
The reproductive potential of a single clone of Pelmatohydra oligactis . 285
WILLIAMS, CARROLL M., AND ROBERT GALAMBOS
Oscilloscopic and stroboscopic analysis of the flight sounds of Drosophila 300
Papers presented at the meeting of the Society of General Physiologists . . . 308
Abstracts of seminar papers presented at the Marine Biological Laboratory. 321
Reports on Lalor Fellowship Research . . . 369

NO. 3. DECEMBER, 1950

BOWDEN, BERNARD J.
Some observations on a luminescent freshwater limpet from New Zealand 373
BURNS, JEAN, AND D. EUGENE COPELAND
Chloride excretion in the head region of Fundulus heteroclitus . . . 381
COSTELLO, DONALD P., AND CATHERINE HENLEY
Heteroploidy in Triturus torosus. II. The incidence of chromosomal variations in shipped larvae . . . 386
DAN, JEAN C.
Sperm entrance in echinoderms, observed with the phase contrast microscope . . . 399
DAN, JEAN C.
Fertilization in the medusan, Spirocodon saltatrix . . . 412
FREEMAN, JOHN A.
Oxygen consumption, brain metabolism and respiratory movements of goldfish during temperature acclimatization, with special reference to lowered temperatures . . . 416
GATES, G. E.
Regeneration in an earthworm, Eisenia foetida (Savigny) 1826. III. Regeneration from simultaneous anterior and posterior transitions . . . 425
GETMAN, HERBERT C.
Adaptive changes in the chloride cells of Anguilla rostrata . . . 439

HASSETT, C. C., V. G. DETHIER, AND J. GANS
A comparison of nutritive values and taste thresholds of carbohydrates for the blowfly 446
KLEINHOLZ, L. H., with the assistance of V. J. HAVEL AND R. REICHART
Studies in the regulation of blood-sugar concentration in crustaceans. II. Experimental hyperglycemia and the regulatory mechanisms 454
REID, W. MALCOLM
Glycogen depletion during starvation in the nemertean, Micrura leidyi (Verrill), and its ecological significance 469
TEWINKEL, LOIS E.
Notes on ovulation, ova, and early development in the smooth dogfish, Mustelus canis 474
WEISEL, GEORGE F.
The comparative effects of teleost and beef pituitary on chromatophores of cold-blooded vertebrates 487

Vol. 99, No. 1 August, 1950

THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

Fifty-Second Report, for the Year 1949—Sixty-Second Year

I. Trustees and Executive Committee (as of August 9, 1949) 1
Standing Committees
II. Act of Incorporation 4
III. By-Laws of the Corporation 4
IV. Report of the Treasurer 6
V. Report of the Librarian 9
VI. Report of the Director 10
Statement 10
Addenda:
1. Memorials 14
2. Appreciation of the services of Mr. Frank M. MacNaught 18
3. The Staff 19
4. Investigators and Students 22
5. The Lalor Fellows 30
6. The Atomic Energy Commission Fellows 31
7. Tabular View of Attendance, 1945–1949 31
8. Subscribing and Cooperating Institutions 31
9. Evening Lectures 32
10. Shorter Scientific Papers (Seminars) 33
11. Members of the Corporation 34

I. TRUSTEES

EX OFFICIO

Lawrason Riggs, *President of the Corporation,* 120 Broadway, New York City
E. Newton Harvey, *Vice President of the Corporation,* Princeton University
Charles Packard, *Director,* Marine Biological Laboratory
Otto C. Glaser, *Clerk of the Corporation,* Amherst College
Donald M. Brodie, *Treasurer,* 522 Fifth Avenue, New York City

EMERITI

G. H. A. Clowes, Lilly Research Laboratory
E. G. Conklin, Princeton University
W. C. Curtis, University of Missouri
B. M. Duggar, University of Wisconsin
W. E. Garrey, Vanderbilt University

Ross G. Harrison, Yale University
F. P. Knowlton, Syracuse University
R. S. Lillie, The University of Chicago
A. P. Mathews, University of Cincinnati
W. J. V. Osterhout, Rockefeller Institute
G. H. Parker, Harvard University

TO SERVE UNTIL 1953

W. R. Amberson, University of Maryland School of Medicine
P. B. Armstrong, Syracuse University
E. G. Ball, Harvard University Medical School
F. A. Brown, Jr., Northwestern University
Robert Chambers, New York University
C. L. Prosser, University of Illinois
A. C. Redfield, Harvard University and Woods Hole Oceanographic Institution
Albert Tyler, California Institute of Technology

TO SERVE UNTIL 1952

E. S. G. Barron, The University of Chicago
D. W. Bronk, Johns Hopkins University
G. Failla, Columbia University
C. O'D. Iselin, Woods Hole Oceanographic Institution
R. T. Kempton, Vassar College
C. W. Metz, University of Pennsylvania
W. R. Taylor, University of Michigan
George Wald, Harvard University

TO SERVE UNTIL 1951

W. C. Allee, The University of Chicago
C. L. Claff, Randolph, Mass.
K. S. Cole, Naval Medical Research Institute
P. S. Galtsoff, U. S. Fish and Wild Life Service
L. V. Heilbrunn, University of Pennsylvania
J. H. Northrop, Rockefeller Institute
H. H. Plough, Amherst College
A. H. Sturtevant, California Institute of Technology

TO SERVE UNTIL 1950

Dugald E. S. Brown, University of Michigan
D. P. Costello, University of North Carolina
M. H. Jacobs, University of Pennsylvania
D. A. Marsland, New York University
A. K. Parpart, Princeton University
Franz Schrader, Columbia University
H. B. Steinbach, University of Minnesota
B. H. Willier, Johns Hopkins University

EXECUTIVE COMMITTEE OF THE BOARD OF TRUSTEES

Lawrason Riggs, *Ex officio, Chairman*
E. N. Harvey, *Ex officio*
D. M. Brodie, *Ex officio*
Charles Packard, *Ex officio*

C. Ladd Prosser, to serve until 1950
H. B. Steinbach, to serve until 1950
C. L. Claff, to serve until 1951
D. A. Marsland, to serve until 1951
Rudolf T. Kempton, to serve until 1952
H. H. Plough, to serve until 1952

The Library Committee

W. R. Taylor, *Chairman*
K. S. Cole
E. N. Harvey
Balduin Lucké
Mary Sears

The Apparatus Committee

A. K. Parpart, *Chairman*
Harry Grundfest
C. L. Prosser
O. H. Schmitt
F. J. M. Sichel

The Supply Department Committee

P. B. Armstrong, *Chairman*
C. L. Claff
P. S. Galtsoff
L. H. Kleinholz
Charles Packard

The Evening Lecture Committee

Charles Packard, *Chairman*
E. S. G. Barron
E. G. Butler

The Instruction Committee

A. K. Parpart, *Chairman*
W. C. Allee
Hope Hibbard
H. H. Plough
Charles Packard, *Ex officio*

The Building and Grounds Committee

C. Lloyd Claff, *Chairman*
W. R. Duryee
Ralph Wichterman

II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Massachusetts, at 11:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held on the second Tuesday in August in each year, at the Laboratory in Woods Hole, Mass., at 10 A.M. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years; and in addition there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk;

(C) Trustees Emeriti, who shall be elected from *present* or *former* Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. Any person interested in the Laboratory may be elected by the Trustees to a group to be known as Associates of the Marine Biological Laboratory.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

The year 1949 was notable from the financial viewpoint because of: 1) the receipt from the Rockefeller Foundation of $150,000 for the restoration and improvement of the "Old Main" laboratory, $25,000 for current expenses, and $4,905.44 in equipment; 2) the gift of a power cruiser, renamed the "Arbacia," from Mrs. John G. Ralston; and 3) the inauguration of the "Frank R. Lillie Memorial Fund" under the able leadership of Dr. G. H. A. Clowes.

As a result of the Rockefeller and other gifts for capital items, the total assets of the Laboratory increased during the year by $173,021.65 to a total of $2,711,621.96.

The current operations for 1949, according to the report of the auditors, Seamans, Stetson and Tuttle of Boston, show total cash receipts of $307,635.14 and disbursements of $310,100.93, the latter including $281,372.89 for current expenses and $28,728.04 for "additions to capital assets" (equipment, etc.). Current income cash on hand December 31, 1949, amounted to $10,290.80. The totals of receipts and disbursements for 1949 were about the same as those for 1948, and this was true for most of the sub-totals for the various departments. One notable exception was the Supply Department where total sales declined from $94,701.77 to $78,770.38. Actual cash receipts in 1949 for the Supply Department were $82,663.36 and expenditures were $82,494.33 showing a net profit of $169.03. These cash figures, however, do not take into account credits from the Instruction and Research Departments for supplies furnished, differences of inventories, depreciation, etc. The complete statement of the auditors shows net income for the Supply Department of $12,573.29.

The Balance Sheet, Statement of Current Surplus, and Summary of Cash Transactions, as given in the auditors' report, follow:

Marine Biological Laboratory Balance Sheet, Dec. 31, 1949

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee		$ 966,928.06	
Securities and Cash in Minor Funds		18,465.54	$ 985,393.60
Plant Assets:			
Land	$ 133,626.38		
Buildings	1,357,661.51		
Equipment	256,825.70		
Library	390,133.36		
	$2,118,246.95		
Less Reserve for Depreciation	774,840.83	$1,343,406.12	
Book Fund, Securities and Cash		5,961.99	$1,349,368.11

Current Assets:

Cash		$ 16,620.21	
Mortgage Note Receivable		2,350.00	
Accounts Receivable		25,111.96	
Inventories:			
Supply Department	$ 52,482.99		
"Biological Bulletin"	16,004.65	$ 68,487.64	
Investments:			
Devil's Lane Property	$ 38,826.68		
Stock in General Biological Supply House	12,700.00		
Other Investment Securities	36,020.00		
Retirement Fund	20,557.08	$ 106,103.76	
Prepaid Insurance		6,665.25	
Items in Suspense (Debits)		1,521.43	
Unadjusted Debits:			
Old Main B'ldg Modernizing:			
Expenditures to Dec. 31	$ 119,279.36		
Cash Unexpended	30,720.64	$ 150,000.00	$ 376.860.25
			$2,711,621.96

Liabilities

Endowment Funds:

Endowment Funds	$ 965,433.05		
Reserve for Amortization	1,495.01	$ 966,928.06	
Minor Funds		18,465.54	$ 985,393.60

Plant Funds:

Mortgage Notes Payable		$ 12,000.00	
Donations and Gifts	$1,181,564.04		
Other Investments in Plant from Gifts and Current Funds	155,804.07	$1,337,368.11	$1,349,368.11

Current Liabilities and Surplus:

Accounts Payable	$ 8,850.56	
Items in Suspense (Credits)	2,566.09	
Current Surplus	215,443.60	
Grant of Rockefeller Foundation to Modernize Old Main Building	150,000.00	$ 376,860.25
		$2,711,621.96

Current Surplus Account

Balance, January 1, 1949		$ 209,432.17
Add:		
Excess of Income over Expense for Year	$ 11,499.89	
Reserve for Depreciation, 1949, Charged to Plant Funds	27,919.94	$ 39,419.83
		$ 248,852.00

Deduct:

Payments from Current Funds during year for Plant Assets:			
Land	$ 3,000.00		
Buildings	11,704.65		
Equipment	11,030.39		
Library	9,673.36		
	$ 35,408.40		
Less Mortgage Note given in Partial Payment for Elliot Property	$ 6,000.00	$ 29,408.40	
Payment on Principal of Mortgage Note given in Partial Payment for "Dolphin"		4,000.00	$ 33,408.40
Balance, December 31, 1949			$ 215,443.60

Summary of Cash Transactions for Year ended Dec. 31, 1949

		Expenditures		
Current Cash	Receipts	Current	Additions to Capital Assets	Total Expenditures
Income from Endowment Funds	$ 40,347.15			
Income from Other Investments	22,159.95			
Real Estate Rentals	7,830.00	$ 2,877.40		$ 2,877.40
Donations	27,602.16			
Membership Dues	2,364.00			
Instruction	12,508.24	9,789.34		9,789.34
Research (including Apparatus and Chemical Departments)	27,127.63	26,091.04	$ 3,361.91	29,452.95
Supply Department	82,663.36	82,215.76	278.57	82,494.33
Mess	40,773.70	38,039.93		38,039.93
Dormitories	19,417.05	10,873.32	6,333.54	17,206.86
"Biological Bulletin"	6,568.78	9,389.46		9,389.46
Library	8,702.67	7,798.35	11,263.01	19,061.36
Book Purchase Account, Woods Hole Oceanographic Institution	1,200.00	1,137.62		1,137.62
Real Estate Sales (received on account)	5,007.00			
Miscellaneous	3,363.45			
Buildings and Grounds		41,558.69	3,491.01	45,049.70
Administration		29,367.71		29,367.71
Interest, Insurance, Cost of Living Bonus, and other Property Maintenance (not allocated)		14,537.82		14,537.82
Payment to Retirement Fund		7,696.45		7,696.45
Payment on Mortgage			4,000.00	4,000.00
	$307,635.14	$281,372.89	$28,728.04	$310,100.93

Cash Balance, January 1, 1949:		
Central Hanover Bank & Trust Co.	$ 5,487.16	
Falmouth National Bank	7,269.43	$ 12,756.59
Receipts		307,635.14
		$320,391.73
Payments		310,100.93
Cash Balance, December 31, 1949:		
Central Hanover Bank & Trust Co.	$ 8,159.11	
Falmouth National Bank	2,131.69	$ 10,290.80

Other Cash Accounts	Balance Jan. 1, 1949	Received	Paid	Balance Dec. 31, 1949
Memhard Fund Cash	$ 33.05	$ 25.00		$ 58.05
Rev. Arsenious Boyer Burse Cash	436.53	125.00		561.53
Lucretia Crocker Fund Cash	1,510.36	357.52	$ 120.00	1,747.88
Bathing Beach (Clowes) Fund	1,000.00			1,000.00
Dr. Frank R. Lillie Memorial Fund Cash	1,000.62	3,951.85		4,952.47
Old Main Building Modernizing Fund Cash		150,000.00	119,279.36	30,720.64
Retirement Fund Cash	1,430.64		5,761.67	4,555.05
Income		1,189.63		
Transfer		7,696.45		
Bio Club Scholarship Fund	187.53	62.50	120.00	130.03
Book Fund	1,416.15		5,702.67	838.93
Income		123.87		
Sale of Securities		5,001.58		
Lalor Foundation Fellowships Cash	878.15	5,000.00	3,759.94	2,118.21

Respectfully submitted,
Donald M. Brodie,
Treasurer

V. REPORT OF THE LIBRARIAN

The sum of $11,164 was appropriated for the year 1949, plus $3000 from the Woods Hole Oceanographic Institution to be applied to staff salaries. The detailed account of the expenditure of the budget is covered in the Treasurer's report. The sum of $1200 was also appropriated by the Woods Hole Oceanographic Institution to cover library acquisitions.

During the year, 1353 (52 new) current journals were received. Of these 417 (11 new) were Marine Biological Laboratory subscriptions, 541 (13 new) were exchanges, and 154 (10 new) were gifts; 66 (2 new) were Woods Hole Oceanographic Institution subscriptions, 158 (13 new) were exchanges and 17 (3 new) were gifts. The noticeable increase in the number of current journals over that for 1948 is due to the fact that the German subscriptions have been resumed—56 out of 129 titles having been re-established since the war.

The Marine Biological Laboratory purchased 72 books, received 11 complimentary copies from authors, 46 gifts from the publishing firms, 37 from the E. L. Mark Library and 26 miscellaneous donations. The Woods Hole Oceanographic Institution purchased 28 titles. A total of 220 books was added to the shelves, the most outstanding purchase being Poggendorff's "Biographisch-literarisches Handwörterbuch" in ten volumes.

There were 9 back sets completed: 2 by purchase (Woods Hole Oceanographic Institution); 6 by exchange (3, Woods Hole Oceanographic Institution) and 1 by gift; 11 were partially completed: 3 by purchase (Woods Hole Oceanographic Institution); 7 by exchange (3, Woods Hole Oceanographic Institution) and 1 by gift.

The reprint additions to the Library numbered 8407. Of these, 1449 were of current issue and 6958 of earlier dates. Gifts from Drs. W. E. Garrey, Mary D. Rogick and Dorothy Wrinch totalled 1208 papers, 394 of which were not already in the Library's collection.

A sum of $5579.61 from the Carnegie Corporation of New York Fund was spent for 8 books, 12 completed back sets, 34 partially completed back sets, and the war-time accumulation of German serials.

Forty-six volumes were borrowed on inter-library loan, and 85 were sent to other institutions. Ninety-seven microfilm orders were filled to the amount of $322.24.

During the year, the sorting of the E. L. Mark Library was completed, and a final report is herewith given: 20,307 reprints were received, 6850 of which were added to the library's collection; 192 books, and 236 serial volumes and numbers. The duplicate material has been placed in the sales-room for clearance.

Grateful acknowledgment is made to Dr. A. W. Pappenheimer for the gift of his valuable collection of reprints. Mr. Henry Stommel's gift of 16 books is also worthy of an expression of appreciation.

The amount of $682.02 was realized from the sales-room. This sum was used to purchase a microfilm camera to replace the old one, which had depreciated to the point of uselessness. The new apparatus has been installed in a recently acquired photography room located next to the librarian's office.

At the end of the year 1949, the Library contained 58,800 bound volumes and 168,935 reprints.

In closing this report, the Librarian wishes to express her gratitude to the members of the Library Committee for their helpfulness throughout the year.

Respectfully submitted,
DEBORAH LAWRENCE,
Librarian

VI. REPORT OF THE DIRECTOR

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY:

Gentlemen:

I submit herewith a report of the sixty-second session of the Marine Biological Laboratory for the year 1949.

1. *Changes in Personnel*

At the Annual Meeting of the Trustees held August 6, 1949, and at the adjourned meeting of August 23rd, resignations were presented by the following:

Lawrason Riggs, President of the Corporation since 1942, and its Treasurer from 1924 to 1942. He stated that rotation in office is desirable, and that since he has served on the Executive Committee for 25 years it was time that he should be replaced.

E. Newton Harvey, Vice President from 1942. Dr. Harvey wished to be relieved of the duties incumbent on this office so that he might devote more time to research.

Charles Packard, Assistant Director and Director since 1937. Having reached the retirement age, he asked that a new Director be appointed in his place.

Frank M. MacNaught, Business Manager since 1913, and already past the age set for retirement, requested that he be retired. An appreciation of his long and devoted service to the Laboratory appears in the appendix of this report.

A Committee, of which Dr. Harvey was Chairman, was appointed to nominate new officers. At the December Meeting of the Trustees, Dr. Philip B. Armstrong was proposed as the new Director and was unanimously elected. The choice is most fortunate. Dr. Armstrong has played an important part in Laboratory affairs for many years, and is thoroughly familiar with the problems with which we are faced. He assumes his duties at a time when plans for developing the resources of the Laboratory are being considered. Under his direction they will be put into operation.

The Executive Committee elected Homer P. Smith as General Manager to replace Mr. MacNaught. He has already served as Assistant Business Manager for three and one-half years.

It is worth noting that within the years 1947–1950 the President, Vice President, Director, Librarian, Manager of the Apparatus Department, Business Manager, and the Superintendent of Buildings and Grounds have been or will be replaced. The terms of office ranged from 8 to 37 years, the average being nearly 20 years.

2. *Organization Problems*

During the summer a Committee, of which Dr. Ball was Chairman, made a thorough study of the increasing activities of the Laboratory, their relationship to each other and to the organization as a whole. The purpose was to establish more clearly than heretofore the lines of authority and responsibility extending from the Executive Committee through the Director and General Manager to the various departments. The recommendations of the Committee were adopted and put into operation with highly satisfactory results.

3. *Increases in Rates and Fees*

A careful analysis of the cost of operating the mess and the residence buildings showed that the rates charged have not been sufficient to cover expenses. The deficits incurred each year had to be paid out of general Laboratory funds. The Executive Committee felt that while research should be subsidized, the cost of running the mess and dormitories should be borne entirely by those who avail themselves of these facilities. The rates were therefore raised by amounts which, it is hoped, will not only wipe out the deficit, but will permit improvements in the service.

4. *Old Main*

The renovation of Old Main, made possible through the generosity of the Rockefeller Foundation, is now complete. An inspection of the building will show how well the Committee in charge of the project, under the energetic Chairmanship of Mr. Claff, has done its work. Careful planning of expenditures permitted the installation of fresh and salt water supplies in every laboratory room, and in addition a new and adequate electric circuit to supply the Mess and other buildings in the area.

5. *Housing*

Many years ago the Laboratory obtained the Gansett and Devil's Lane tracts to provide its members with suitable building lots. In the former area all the lots have now been sold. In Devil's Lane 23 lots have been purchased and seven houses are either under construction or already completed. Others will be built in the near future. The Town of Falmouth has taken over the roads which had been laid out, and will be responsible for their maintenance. Water mains have also been laid, and electricity will be available in the summer of 1950.

6. *Gifts*

The Laboratory acknowledges with gratitude the gift of a boat, presented by Mrs. John G. Ralston of Dixon, Illinois. This craft, now appropriately named "Arbacia," has been equipped with light dredging gear and will be used in a biological survey of this area. It is fast, seaworthy, and can accommodate a crew which may find it necessary to spend several days away from Woods Hole on extended collecting trips.

Dr. William D. Curtis gave $400 to be used for elevating the roof of the pilot house on the "Dolphin."

The Associates of the Marine Biological Laboratory contributed $1,370 for the purchase of research apparatus.

The Rockefeller Foundation gave $4,905.44 to purchase equipment to be used by the Institute of Muscle Research under the direction of Dr. Szent-Gyorgyi.

7. *The F. R. Lillie Memorial Research Fund*

The appeal for contributions to the F. R. Lillie Memorial Research Fund has resulted in a generous response from members of the Corporation and from friends. In addition, Dr. Clowes, Chairman of the Committee in charge of the drive, has been successful in obtaining substantial gifts. Donations received from members will be used for the erection of a bronze tablet to be placed in the lobby of the Brick Building. Together with the present Agassiz and Whitman tablets it will be a part of a group which will emphasize the service of these three great scientists in the initiation, direction, and development of the Marine Biological Laboratory. The larger part of the fund will be used for general research purposes, including the biological survey.

8. *The Biological Survey*

The need for a new biological survey of this region has been repeatedly emphasized in recent years. Plans for a long range project are under consideration, but in the meantime, the finding of adequate sources of sea urchins is of immediate practical importance. This was shown by the experience of last summer when, for a time, no urchins could be obtained. Under the direction of Mr. McInnis, Vineyard Sound will be thoroughly explored. Later, the area to the westward will be worked over. The results of these operations are shown on a chart in the Committee Room. Our Naturalist, Dr. Rankin, has been placed in charge of the more general survey.

9. *Instruction*

Dr. Frank A. Brown, Jr., resigned after completing five years of most successful service as head of the course in Invertebrate Zoology. Under his direction the present staff, with some assistance from former staff members and others, have published a laboratory guide, "Selected Invertebrate Types." The book is extensively illustrated, and in condensed form brings together the content of many monographs, and other material not in monograph form. In the introduction Dr. Brown expresses the hope, in which we all share, that "the publication of the volume will result in a rejuvenation in the teaching of this important subject which provides so much promise in the solution of virtually every basic problem in biology and medicine."

Dr. Lewis H. Kleinholz, Professor of Biology at Reed College, was appointed to take charge of the course in place of Dr. Brown.

10. *Deaths*

The Laboratory has lost one of its most distinguished members, Dr. Leonor Michaelis, whose scientific contributions based on work done here, added much to the fame of this institution. His lucid discussions at the Friday Evening Lectures and before the Physiology Class will be long remembered.

11. *Election of Trustees*

At the Annual Meeting of the Corporation held August 9, 1949, the following were elected Trustees of the Marine Biological Laboratory:

8 Trustees—Class of 1953

W. R. Amberson
P. B. Armstrong
E. G. Ball
F. A. Brown, Jr.
R. Chambers
C. L. Prosser
A. C. Redfield
A. Tyler

12. *There are appended as parts of this report:*

1. Memorials
2. Appreciation of the services of Mr. Frank M. MacNaught
3. The Staff
4. Investigators and Students
5. The Lalor Fellows
6. The Atomic Energy Fellows
7. Tabular View of Attendance, 1945–1949
8. Subscribing and Cooperating Institutions
9. Evening Lectures
10. Shorter Scientific Papers presented at the Seminars
11. Members of the Corporation

Respectfully submitted,

CHARLES PACKARD,
Director

1. MEMORIALS

Sumner Cushing Brooks, 1888–1948

By

H. F. BLUM

In the death of Sumner Cushing Brooks in Bermuda on April 23rd, 1948, the Marine Biological Laboratory lost a staunch friend as well as a distinguished scientist. His unflagging interest in the laboratory is testified to by the regularity of his summer attendance here, even after he took up his residence in 1928 on the other side of the continent. His first summer as independent investigator was in 1919, his last in 1946, although he paid a brief visit here in 1947. His wife, Matilda Moldenhauer Brooks, usually accompanied him, sharing a laboratory in which they worked on problems often closely allied. Brooks became a member of the Corporation in 1923, and was elected a Trustee in 1941.

Although born in Japan, Brooks could claim only very short residence there. The accident of his birthplace resulted from his father's position as Professor at the Imperial College of Agriculture, which he relinquished a few months after Sumner's birth to accept a professorship at Massachusetts Agricultural College.

Sumner Brooks took his bachelor's degree at the latter institution in 1910, and remained there afterwards as an assistant in Botany. He obtained his Ph.D. in the same subject from Harvard in 1916. Subsequently he held various positions, including six years in the Hygienic Laboratory of the U. S. Public Health Service, and professorships at Bryn Mawr, Rutgers, and California.

Sumner Brooks loved to travel, and this urge took him to many parts of the earth. Often his journeys had as their chief aim, experimental studies which could best be carried on where the particular biological material was available; but wherever he went he was an avid student of nature and a careful observer of whatever came within his view, particularly bird life. I remember, for example, his account of timing the wing beats of albatross and correlating this with latitude as he came home from the South Seas to Berkeley. Such observations were seldom if ever published—this was pure avocation, stemming from his keen enjoyment of nature. His interest in birds was with him when he moved from the east coast to the west, where he was soon associated with an active group of ornithologists, and at the time of his death he was an officer in the Cooper Ornithological Society.

Brooks' scientific achievements, particularly in the field of permeability, need no review for this audience. His researches were always attempts to probe into fundamental problems of cellular physiology, rather than applications of existing biological knowledge. He was a general physiologist in the broadest sense, and when the formation of a Society of General Physiologists was undertaken here, in 1946, he, of course, took an active part.

Although suffering severe physical handicaps during the last years of his life, he continued until the end his interest in biology inside and outside the laboratory. His death came quickly, and as he might have wished—during a period of investigation, characteristically far from home.

Selig Hecht, 1892–1947

By

OTTO GLASER

Selig Hecht arrived at Woods Hole in 1918. It was five years after his graduation from the City College of New York, where he had studied chiefly Physics, Chemistry, and Mathematics, but where also, and with rare perception, George Gilmore Scott had interested his pupil in measurements on dogfish. Two seasons at Beaufort, 1912 and 1913, produced additional measurements on 12 species of teleost. Perhaps most decisive at this stage of Hecht's career was the display of mathematically definable order by quantitative data on various dimensions of an organism and the obvious relevance of the measurements to one another. In 1916 a third paper, "Form and Growth of Fishes," comments on the use of volumes and especially weights as criteria of growth and demonstrates how fluent he had become in the idiom of biology. "The usefulness of an organ," he wrote, "and the adaptedness of an organism to its environment, are hardly functions of their weight. The form, however, is of prime importance. Sixteen years later this work was assigned its place in what is now called Allometry.

Before entering Harvard in 1915, Hecht served as a Pharmacologist in the department of Agriculture and in one year saved enough money to begin graduate study. A doctoral thesis prepared under George Howard Parker took Hecht to Bermuda. "The Physiology of *Ascidia atra*" was published in 1918, part of the thesis having won the Bowdoin Prize in 1917 "for essays of high literary merit." These observations and experiments included, incidentally, the qualitative basis for all his later work.

Fresh from a rigorous quantitative analysis of photic responses in *Ciona intestinalis*, studied at La Jolla, Hecht knew precisely why he came to Woods Hole. He had postulated a simple mechanism. A photo-sensitive material is present in the Ciona siphons. Under the influence of light this substance breaks down into two others whose reunion restores the original photosensitive material. Here was the first version of a model destined for some changes and numerous applications.

Between 1918 and 1924, the year of his election to our Corporation, Hecht tested this hypothesis with the utmost care on the clam, *Mya arenaria*. From conversations and the nature of the experiments on Mya, it was apparent that he expected to study vision, but also that he intended to rest his case among organisms where photoreception is uncomplicated by psychological factors or even optics. About sixteen publications dealt with the duration of the latent period; with the stationary state; with intensity and photoreception; with the kinetics of dark adaptation; with time, intensity, and wave length, in photic excitation; and with the applicability of the Bunsen-Roscoe law to minimal and other energy levels.

During this period Hecht labored in the catacombs then under Old Main and frequently by accident but sometimes to relieve his maddening migraine, whacked his head against the rafters. In those days he spoke poignantly about three books that had captivated his imagination. One was George Moore's "The Brook Kerith"—reread several times and always with tears over the same passages. But there were also the concise "Quantitative laws in biological chemistry" by Arrhenius, and Mellor's "Higher mathematics for students of chemistry and physics." From Arrhenius he absorbed the idea of using temperature as a tool for the interpretation of biological processes in terms of chemical reaction kinetics. From Mellor and more specialized sources he acquired the technique and insights that enabled him to extract meaning, implications and the implications of these, from his meticulous data and his meticulously drawn curves. A curve was more than a graphic representation. It was something to "stew" over until its naive statements and their direct and indirect consequences crystallized into a system internally consistent and congruous with other data, either known or obtainable by further experimentation. In the atmosphere of that decade, facile taxonomists classified Hecht's rationalism and sophisticated methodology mostly as witchcraft.

By 1921 a fourth year had rolled by as Assistant Professor of Biochemistry in the Creighton University Medical School in Omaha, Nebraska. Seconded by two powerful friends, Jacques Loeb and Thomas Hunt Morgan, Hecht then embarked on a series of National Research and General Education Board Fellowships. For another five years these enabled him to work now with Henderson at Harvard, now with Baly or Barcroft in England; to travel on the continent, and to spend a year at Naples. By 1926 he was appointed Associate Professor of Biophysics at Columbia and two years later rose to full professorship. After 1924 he did not return to Woods Hole until 1927 and after 1929 came only for short visits. Between these last dates he worked in room 231 of the Brick Building and devoted his time largely to the effects of intermittent light; produced his theoretical derivation of Talbot's Law, and a validation of the theory on Mya.

Hecht's entire scientific development is an illustration of intellectual orthogenesis. Even before his foundations had solidified in detail, he used Ciona and Mya as guides to dark adaptation and intensity discrimination in man. His prolonged and incomparable exploration of the human retina began in 1920. These investigations quickly uncovered objective criteria for distinguishing rod from cone vision. His dramatic demonstration that curves depicting the relation between intensity and visual acuity are population curves reflecting differences in the sensitivity of individual rods and cones, and also the number of such receptors active at given intensities, was presented in a memorable Friday Evening lecture in 1924. Later the underlying idea was tested more directly on the eyes of fruit flies and bees where the same relations hold and where the number of functional ommatidia can be easily counted.

Beginning also in 1920, and interspersed with the researches of the next 22 years, were experiments on the photochemistry of Visual Purple. Here temperature effects on the rate of bleaching indicated a first order reaction directly proportional to intensity and with no thermal increment. With the assistance of Chase, Schlaer and Haig this phase culminated in the verification, long overdue, of Kühne's reported regeneration of Visual Purple in solution. The Ciona model had found its replica in the vertebrate eye.

From the outset Hecht brooded over the perennially difficult problems of color vision, but felt unprepared. In another 12 papers he reexamined every relevant aspect of his foundations. At Naples in 1925, he reinvestigated Ciona and added the lamellibranch Pholas to his small repertoire of animals. He was now postulating for Ciona, Mya and Pholas photoreceptive processes identical in architecture but different in their chemical components.

The frontal attack on Color Vision began in 1928. Characteristically, Hecht established almost at once the essential formal adequacy of Young's three-fiber theory. This was indeed capable of yielding a quantitative formulation of Color Vision. Nevertheless, retaining only the structural intuition in Young's view and integrating this with the Helmholtz three-substance theory, he decided in favor of a three-cone hypothesis. This became basic for his analysis of the several types of color-blindness.

After night-blindness had been associated by others with deficiency or absence of Vitamin A, and Wald had identified both Vitamin A and the carotenoid retinene in normal retinas, Hecht instigated five special studies on dark adaptation and from the general similarities between rod and cone vision, indicated that Vitamin A is probably present also in cones. In cooperation with Mandelbaum, a study of Vitamin A and Rod-Cone adaptation in cases of cirrhosis of the liver was followed by the development of a diagnostic method for determining Vitamin A deficiencies.

Early in the Mya era, Hecht had dealt with minimal responses in relation to minimal energy levels. Twenty years later, after a study of night vision in the owl, he undertook from an entirely different standpoint his Quantum Analysis of Vision. Given the discontinuities of light, the discontinuities of the retinal receptors, and certain ingenious corrections for reflection and absorption by the non-photosensitive ocular media, he succeeded in finding what proportion of incident light actually reaches the retina. In order to see, a minimum of from 5 to 14 rods must each absorb one quantum of light. At this level he maintained conventional explanations for variability of behavior breakdown for here the stimulus is less accurately controlled than the organism.

Hecht's output includes about twenty-two communications of a general nature. Some of these, notably his first papers on temperature, on Young's theory, and his derivation of Talbot's Law, suggest the moods of the theoretical physicist. About fifteen others are lectures, contributions to symposia, critical summaries and reviews. In this family of papers, Hecht kept up with himself, tightening his hold by keeping all parts of his field freshly before his mind.

The audiences he addressed differed widely. Some were essentially lay; others were Ophthalmologists, Clinicians, Physicists, Chemists, or Biologists. Mostly in English, the list also includes German, French and even Russian items. The longest, with 47 illustrations, is the great Summary in the *Asher-Spiro Ergebnisse* for 1931, which appeared later in book form in French. Repetitions were, of course, unavoidable and on occasion he followed verbatim earlier statements that he could not improve. Yet, in general, each attempt to reach a different group involved a fresh effort at simplification and intelligibility. Concerned always with the historical development of his subject he frequently referred to contributions over 100, and even 226 years old. From the Classics, especially Koenig and Brodhun, 1889, he recovered usable measurements not influenced by his own ideas and hence sources of the greatest satisfaction. Unless editorial policies over-ruled, his bibliographies were unscathed by the drought that dries the streamlined reference lists. He always gave the full title of every citation.

With our entry into the war the practical value of Hecht's experience became at once obvious. Under the Division of Medical Sciences of the National Research Council, he joined the sub-committee on Visual Problems and under the O.S.R.D. became an official investigator both for the Army and the Navy. Promptly Hecht and his colleagues were swamped by Government contracts entailing special laboratory and field investigations. More grinding experiences were provoked by the paper work, and the innumerable discussions with Officers of the Armed Forces. When not flying to Camp LeJeune in North Carolina or to Milwaukee, Hecht oscillated between Bridgewater, Connecticut, and New York or between New York and Washington. Only a few of the results have been printed, *e.g.*, on the relation between Anoxia and Brightness Discrimination; on size, shape, and contrast in the Detection of Targets by Daylight and on Dark Adaptation following Light Adaptation to Red and White Lights. To this period belongs also a popular article in Harper's magazine on "Seeing in a black out."

For Biology in general, it is unfortunate that the four large volumes of the Hechtian Corpus should be almost exclusively the possession of specialists. Here we have one of the best examples to show how a limited acreage cultivated intensively may produce flowers of research able to pollinate adjacent fields and also to set the fruits required for self-propagation or available for immediate consumption in cases of need. More widely appreciated, the logical sequence of these investigations and the special logic of each installment, could only raise the level of scientific writing. His style permitted revealing comments and asides. "The essential function of vision is to explore the environment by the use of light"; "My purpose is not to add to the literature but rather to subtract from it"; "Experiments are exciting in proportion to the ideas which determine their existence"; "The ideas themselves must be considered merely as quantitative experiments in thinking about Color Vision," are all trenchant sentences. Hecht unmasked more completely during a joint meeting of the Optical Society and the Society of Rheology. In reporting his Quantum Studies he begins: "I would like to lay before you more than the data and conclusions. If possible I want to convey something of the pleasure with which this research has been associated, something of the intellectual excitement which surrounded its progress. Researches vary in this respect but of all those in which I have engaged none has carried such a consistent accompaniment of delight and I hope some of this may be carried over in the presentation."

All his writings unfold the teacher who could take his learning lightly yet with no trace of flippancy, or clowning. We recall a charming but profound essay on Human Behavior and the Uncertainty Principle printed in Harper's Magazine and editorialized in the New York Times. Certainly "Explaining the atom" is another case in point. The most satisfactory of a group of popular books on atomic energy, it had a surprising sale, was adopted as a text by the Armed Forces and explains his presence on the Committee of Atomic Scientists.

In conventional teaching he was extraordinarily effective. The impressionism that gave such flare to hundreds of his watercolors bespoke a deep seated temperamental diathesis. In science, too, he could hack a trail through jungles of detail and irrelevancies, irksome, but not invariably as innocent as he thought. Always he reached a clearing. At the New School for Social Research he impressed Alvin Johnson as the best teacher he had ever known.

Hecht was one of those about whom the hours seem to hover in their flight. Preoccupied as he was, he always had time to paint and sing; time for literature, concerts, and conversations; time also to run a summer school for children who repeated the classical experiments of Archimedes, Galileo and Newton in a Vermont barn with the simplest of improvised equipment. Of himself he gave lavishly. Criticisms, suggestions, help with the experiments of others and with mathematical interpretations were available in inexhaustible supply. He conducted a lively correspondence. Still his energies were unspent. He revived and edited the Columbia Biological Series. His services on the Editorial Boards of the *Biological Bulletin*; of the *Journal of the Optical Society of America*; of the *Monographs of Experimental Biology*; and of the *Documenta Ophthalmologica* were additional outlets to regulate the creative pressure.

It is difficult to realize that one who was to cherish the Ives Medal of the Optical Society, the Townsend Harris Medal of the City College of New York and election to the National Academy of Sciences could, in 1917, find no proper niche for his unique constellation of talents. Are there comparable instances today? During the decade between the doctorate and his Professorship, he depended heavily on a few friends and on the constant sympathy, encouragement and sacrifices of his wife, Celia Hecht. Why were these the only human beings to recognize the early symptoms of his great potentialities? That one so vital could be struck down with all his powers still at flood tide remains almost incredible to those who, in fancy, can still hear these corridors reverberate with his impassioned argument and his Homeric laughter.

2. In Appreciation

The following minute, prepared by Dr. H. H. Plough, was adopted by the Trustees at the meeting held in New York City on December 29, 1949:

On January 1, 1950, Frank M. MacNaught will retire as business manager of the Marine Biological Laboratory. When he thus attains *emeritus* status, Mr. MacNaught will have served the laboratory for thirty-seven years in what is in many respects the most important position on the laboratory staff. During that period he has come to know more members and guests of the laboratory than any other officer, excepting only his chief associate, Miss Polly Crowell. There are none of this long list but bear for Mr. MacNaught not only respect but warm personal regard.

Mr. MacNaught was appointed as the sole member of the business staff in 1913. The writer of this minute was a student in one of the courses in that summer, and he still remembers with pleasure the kindness and friendliness shown by Mr. MacNaught to a young college student who was unfamiliar with the arrangements at the laboratory. His unique gift of friendliness, his ability to treat each member of the laboratory as though his needs were paramount, his willingness to spend time with each one personally until, within the range of the possible, his needs were satisfied—these human qualities have endeared Mr. MacNaught to hundreds of Woods Hole visitors. They will hope to see him each summer even if he turns over his official duties to younger hands.

The work of Mr. MacNaught rapidly grew in the years following his appointment. During the term of Frank R. Lillie as director, it was realized that two sorts of service were required in the office and he took them both over. One had to do, as originally contemplated, with keeping the accounts of the laboratory under the advice of the Director for consideration of the Executive Committee. In another respect, however, service as registrar was required—including not only arrangements for laboratory space and course tables, but the even more difficult responsibility of arranging and assigning housing, and of taking responsibility for the mess and its many temporary employees. All of these many tasks were gradually consolidated in Mr. MacNaught's capable hands and he was, by appointment of the Executive Committee, made Business Manager in 1916.

Since then he has served under two directors following Dr. Lillie—Drs. Jacobs and Packard, and has continued to merit the confidence and cooperation of each.

Both Trustees and Staff congratulate Mr. MacNaught on a unique and valuable contribution to the Marine Biological Laboratory, and hope they may continue to call him fellow member and friend.

For the Trustees,
WILLIAM R. AMBERSON,
Secretary of the Board

3. THE STAFF, 1949

CHARLES PACKARD, Director, Marine Biological Laboratory, Woods Hole, Massachusetts.

SENIOR STAFF OF INVESTIGATION

E. G. CONKLIN, Professor of Zoology, *Emeritus,* Princeton University.
W. E. GARREY, Professor of Physiology, *Emeritus,* Vanderbilt University Medical School.
RALPH S. LILLIE, Professor of General Physiology, *Emeritus,* The University of Chicago.
A. P. MATHEWS, Professor of Biochemistry, *Emeritus,* University of Cincinnati.
G. H. PARKER, Professor of Zoology, *Emeritus,* Harvard University.

ZOOLOGY

I. CONSULTANTS

LIBBIE H. HYMAN, American Museum of Natural History.
A. C. REDFIELD, Woods Hole Oceanographic Institution.

II. INSTRUCTORS

F. A. BROWN, JR., Professor of Zoology, Northwestern University, in charge of course.
W. D. BURBANCK, Professor of Biology, Drury College.
C. G. GOODCHILD, Professor of Biology, S.W. Missouri State College.
L. H. KLEINHOLZ, Associate Professor, Reed College.
JOHN H. LOCHHEAD, Assistant Professor of Zoology, University of Vermont.
MADELENE E. PIERCE, Associate Professor of Zoology, Vassar College.
W. M. REID, Professor of Biology, Monmouth College.
T. H. WATERMAN, Assistant Professor in Biology, Yale University.

III. LABORATORY ASSISTANTS

R. S. HOWARD, University of Miami.
MARIE WILSON, Northwestern University.

EMBRYOLOGY

I. Instructors

Donald P. Costello, Professor of Zoology, University of North Carolina, in charge of course.
William W. Ballard, Professor of Zoology, Dartmouth College.
Arthur L. Colwin, Assistant Professor of Zoology, Queens College.
Charles B. Metz, Assistant Professor of Zoology, Yale University.
James A. Miller, Associate Professor of Anatomy, Emory University.
Albert Tyler, Associate Professor of Embryology, California Institute of Technology.

II. Research Assistant

Margaret E. M. Davidson, McGill University.

III. Laboratory Assistants

Helen A. Padykula, Mount Holyoke College.
James M. Moulton, Harvard University

PHYSIOLOGY

I. Consultants

Eric G. Ball, Professor of Biochemistry, Harvard University Medical School.
Merkel H. Jacobs, Professor of Physiology, University of Pennsylvania.
Otto Loewi, Professor of Pharmacology, New York University, School of Medicine.
Arthur K. Parpart, Professor of Biology, Princeton University.

II. Instructors

E. S. Guzman Barron, Associate Professor of Biochemistry, The University of Chicago, in charge of course.
M. J. Kopac, Associate Professor of Biology, New York University.
Hans Neurath, Professor of Biochemistry, Duke University Medical School.
Robert F. Pitts, Professor of Physiology, Syracuse University, College of Medicine.
H. Burr Steinbach, Professor of Zoology, University of Minnesota.
George Wald, Professor of Biology, Harvard University.

BOTANY

I. Consultants

Bostwick H. Ketchum, Woods Hole Oceanographic Institution.
Wm. Randolph Taylor, Professor of Botany, University of Michigan.

II. Instructors

Maxwell S. Doty, Assistant Professor of Botany, Northwestern University. In Charge of Course.
Edwin T. Moul, Instructor in Botany, Rutgers University.
R. D. Wood, Assistant Professor of Botany, Rhode Island State College.

III. Research Assistant

Justine Garnic, Carnegie Institute of Technology.

IV. Laboratory Assistant

Albert J. Bernatowicz, University of Michigan.

V. Lecturers

J. B. Lackey, Philadelphia, Pa.
Ruth Patrick, Curator of Limnology, Academy of Natural Sciences of Philadelphia.

VI. Field Consultant and Collector

Hannah T. Croasdale, Research Associate, Dartmouth College.

EXPERIMENTAL RADIOLOGY

G. Failla, College of Physicians and Surgeons, Columbia University.
L. Robinson Hyde, Phillips Exeter Academy, Exeter, N. H.

LIBRARY

Deborah Lawrence, Librarian
Margaret P. McInnis
Mary A. Rohan
Jean Goodfellow

APPARATUS DEPARTMENT

Robert B. Mills, Manager
J. D. Graham
Dorothy LeFevre
E. P. Little, Consultant
Seaver R. Harlow, Machinist

CHEMICAL DEPARTMENT

Robert B. Mills, Manager

SUPPLY DEPARTMENT

James McInnis, Manager
John S. Rankin, Naturalist
Ruth Crowell
Marcia McLaughlin
M. B. Gray
A. M. Hilton
W. E. Kahler
Carl O. Schweidenback
G. Lehy
R. E. Tonks
F. N. Whitman

GENERAL OFFICE

F. M. MacNaught, Business Manager
Homer P. Smith, Assistant Business Manager
Polly L. Crowell
Mrs. Lila S. Myers
Norma Ferreira

GENERAL MAINTENANCE

R. W. Kahler, Manager

Robert Adams
R. Gunning
J. H. Head
G. A. Kahler
A. Neal
G. T. Nickelson, Jr.
A. J. Pierce
T. E. Tawell

4. Investigators and Students

Independent Investigators, 1949

Abrams, Richard, Assistant Professor, University of Chicago.
Allen, Ezra, Visiting Professor of Biology, Stetson University.
Allen, M. Jean, Instructor in Zoology, University of New Hampshire.
Alscher, Ruth P., Instructor, Manhattanville College.
Amberson, William R., Professor of Physiology, University of Maryland Medical School.
Anderson, Rubert S., Professor of Physiology, University of South Dakota.
Atwood, Kimball C., Research Associate in Microbiology, Columbia University.
Baily, Norman A., Research Scientist, Columbia University.
Ball, Eric G., Professor of Biological Chemistry, Harvard Medical School.
Ballard, W. W., Professor of Biology, Dartmouth College.
Barron, E. S. Guzman, Associate Professor of Biochemistry, The University of Chicago.
Berger, Charles A., Director, Biological Laboratory, Fordham University.
Bernstein, Maurice H., Graduate Assistant, Washington University.
Bliss, Alfred F., Associate Professor of Physiology, Tufts College Medical School.
Blum, Harold F., Physiologist, Princeton University.
Bridgman, Anna J., Professor of Biology, Limestone College.
Brooks, Matilda M., Research Associate in Biology, University of California.
Brown, Frank A., Jr., Professor of Zoology, Northwestern University.
Browning, Iben, National Research Fellow, University of Pennsylvania.
Brust, Manfred, Research Assistant in Physiology, University of Chicago.
Burbanck, W. D., Chairman, Department of Biology, Drury College.
Butler, Elmer G., Professor of Biology, Princeton University.
Chase, Aurin M., Associate Professor of Biology, Princeton University.
Cheney, Ralph H., Professor of General Physiology, Brooklyn College.
Claff, C. Lloyd, Research Associate in Surgery, Harvard Medical School.
Clark, Arnold M., Professor of Biology, University of Delaware.
Clark, Eliot R., Professor of Anatomy, University of Pennsylvania School of Medicine.
Clark, Leonard B., Chairman, Department of Biology, Union College.
Clement, A. C., Professor of Biology, College of Charleston.
Clowes, G. H. A., Research Director *Emeritus,* Lilly Research Laboratories.
Cohen, Arthur I., Teaching Assistant in Zoology, University of Minnesota.
Cohen, Isadore, Associate Professor, American International College.
Cole, Kenneth S., Scientific Director, Naval Medical Research Institute.
Colwin, Arthur L., Assistant Professor of Biology, Queens College.
Conklin, E. G., Professor of Biology *Emeritus,* Princeton University.
Cooperstein, Sherwin J., Instructor in Anatomy, Western Reserve University.
Costello, Donald P., Professor of Zoology, University of North Carolina.
Cotzias, George C., Assistant Physician, Rockefeller Institute.
Coyle, Elizabeth E., Associate Professor of Biology, College of Wooster.
Croasdale, Hannah, Associate in Zoology, Dartmouth College.
Curtis, W. C., Professor of Zoology *Emeritus,* University of Missouri.
Dahl, A. Orville, Chairman, Department of Botany, University of Minnesota.
DeLamater, Edward D., Associate Research Professor of Dermatology, University of Pennsylvania.
Dent, J. N., Associate Professor of Biology, University of Virginia.
Diller, Irene C., Research Cytologist, Institute for Cancer Research.
Diller, William F., Assistant Professor of Zoology, University of Pennsylvania.
Dixon, June A., Graduate Assistant in Zoology, Washington University.
Doty, Maxwell S., Assistant Professor of Biology, Northwestern University.
Driscoll, Dorothy H., Instructor, Smith College.
Duryee, William R., Cytologist, Carnegie Institution.
Failla, G., Professor of Radiology, Columbia University.
Ferguson, Frederick P., Assistant Professor of Physiology, University of Maryland Medical School.

Fogelman, M. J., Fellow in Neurosurgery, Southwestern Medical College.
Frenkel, Albert W., Assistant Professor of Botany, University of Minnesota.
Gaffron, Hans, Associate Professor of Biochemistry, University of Chicago.
Garrey, W. E., Professor of Physiology *Emeritus,* Vanderbilt University School of Medicine.
Gilman, Lauren C., Associate Professor of Zoology, University of Miami.
Glaser, Otto C., Professor of Biology, Amherst College.
Goodchild, Chauncey G., Professor of Biology, South West Missouri State College.
Gould, Harley N., Professor of Biology, Newcomb College, Tulane University.
Grand, C. G., Research Associate, New York University.
Green, James W., Assistant Professor of Physiology, Rutgers University.
Greenberg, Ruven, Instructor of Physiology, Ohio State University.
Grosch, Daniel S., Assistant Professor of Zoology, North Carolina State College.
Grundfest, Harry, Associate Professor of Neurology, Columbia University.
Harris, Daniel L., Assistant Professor of Physiology, University of Chicago.
Harvey, Ethel Browne, Independent Investigator of Biology, Princeton University.
Harvey, E. Newton, Professor of Physiology, Princeton University.
Haxo, Francis, Instructor in Biology, Johns Hopkins University.
Haywood, Charlotte, Professor of Physiology, Mount Holyoke College.
Heidenthal, Gertrude, Associate Professor of Biology, Russell Sage College.
Heilbrunn, L. V., Professor of Zoology, University of Pennsylvania.
Henley, Catherine, Research Assistant, University of North Carolina.
Hickson, Anna Keltch, Research Chemist, Lilly Research Laboratories.
Hodes, Robert, Professor of Experimental Neurology, Tulane University School of Medicine.
Hopkins, Hoyt S., Associate Professor of Physiology, New York University.
Hsiao, Sidney C., Guest Professor of Biology, New York University.
Hunter, F. R., Associate Professor of Physiology, University of Oklahoma.
Irving, Laurence, Consultant, U. S. Public Health Service.
Jacobs, M. H., Professor of General Physiology, University of Pennsylvania.
Jenkins, George B., Professor of Anatomy *Emeritus,* George Washington University.
Jepps, Margaret W., Glasgow University.
Kaan, Helen W., Research Associate, National Research Council.
Kempton, Rudolf T., Professor of Zoology, Vassar College.
Keston, Albert S., Assistant Professor of Chemistry, New York University.
Kind, C. Albert, Assistant Professor of Chemistry, University of Connecticut.
Kisch, Bruno, Professor of Chemistry, Yeshiva University.
Kitchin, I. C., Associate Professor of Biology, University of Georgia.
Kleinholz, Lewis H., Associate Professor of Biology, Reed College.
Klotz, I. M., Associate Professor of Chemistry, Northwestern University.
Krahl, Maurice E., Associate Professor of Biochemistry, Washington University.
Kuffler, Stephen W., Assistant Professor of Physiological Optics, Johns Hopkins Medical School.
Lajtha, Abel, Institute for Muscle Research.
Lancefield, Rebecca, Associate Member, Rockefeller Institute.
Lansing, Albert I., Associate Professor, Washington University School of Medicine.
LeFevre, Paul G., Assistant Professor of Physiology, University of Vermont College of Medicine.
Levy, Milton, Associate Professor of Chemistry, New York University College of Medicine.
Lillie, Ralph S., Professor of Physiology *Emeritus,* University of Chicago.
Lochhead, John H., Assistant Professor of Zoology, University of Vermont.
Lovelace, Roberta, Adjunct Professor of Biology, University of South Carolina.
Lucke, Balduin, Professor of Pathology, University of Pennsylvania.
Luyet, B., Professor of Biophysics, Saint Louis University.
Marmont, George H., Assistant Professor of Physiology, University of Chicago.
Marshak, A., Research Associate, New York University College of Medicine.
Marsland, Douglas, Professor of Biology, Washington Square College.
Matoltsy, Alexander G., Institute for Muscle Research.
Matzke, Edwin B., Professor of Botany, Columbia University.

Mazia, Daniel, Professor of Zoology, University of Missouri.
McColl, J. D., University of Western Ontario Medical School.
Menkin, Valy, Associate Professor of Experimental Pathology, Temple University Medical School.
Metz, Charles B., Assistant Professor of Zoology, Yale University.
Mihalyi, Elemer, Institute for Muscle Research.
Miller, James A., Associate Professor of Anatomy, Emory University.
Milne, Lorus J., Associate Professor of Zoology, University of New Hampshire.
Moore, George M., Associate Professor of Zoology, University of New Hampshire.
Moul, Edwin T., Assistant Professor of Botany, Rutgers University.
Musacchia, X. J., Instructor, Saint Louis University.
Nachmansohn, David, Assistant Professor of Neurology, Columbia University.
Nelson, Leonard, Instructor in Zoology, University of Nebraska.
Neurath, Hans, Professor of Physical Biochemistry, Duke University School of Medicine.
Noland, Jerre L., Research Assistant in Biochemistry, University of Wisconsin.
O'Brien, John A., Assistant Professor of Biology, Catholic University of America.
O'Brien, John P., Assistant Professor of Zoology, Marquette University.
Osterhout, W. J. V., Member *Emeritus,* Rockefeller Institute.
Palay, Sanford L., Instructor in Anatomy, Yale University School of Medicine.
Parmenter, Charles L., Professor of Zoology, University of Pennsylvania.
Parpart, Arthur K., Chairman, Department of Biology, Princeton University.
Parshley, Howard M., Professor of Zoology, Smith College.
Pick, Joseph, Associate Professor in Anatomy, New York University College of Medicine.
Pierce, Madelene E., Associate Professor of Zoology, Vassar College.
Plough, Harold H., Professor of Biology, Amherst College.
Plummer, Jewel I., Teaching Fellow, New York University.
Prosser, C. Ladd, Professor of Zoology, University of Illinois.
Provasoli, Luigi, Research Associate, Haskins Laboratories.
Reid, W. Malcolm, Head of the Department of Biology, Monmouth College.
Reiner, John M., Research Associate, Tufts College Medical School.
Reinhard, E. G., Head of the Department of Biology, Catholic University of America.
Rieser, Peter, University of Pennsylvania.
Rose, S. Meryl, Associate Professor of Zoology, Smith College.
Rosenthal, Theodore B., Research Associate, Washington University Medical School.
Rossi, H. H., Physicist, Department of Radiology, Columbia University.
Roth, Jay S., Assistant Professor of Biochemistry, Rutgers University.
Rothenberg, Mortimer A., Columbia University.
Roy, S. S., Lecturer, Calcutta University.
Rudenberg, Frank H., Harvard University.
Rugh, Roberts, Associate Professor of Radiology, Columbia University.
Sarkar, Nirmal, Lecturer in Chemistry, Calcutta University.
Schaeffer, A. A., Professor of Biology, Temple University.
Schallek, William B., Assistant Professor of Biology, University of Oregon.
Schmitt, Francis O., Head of the Department of Biology, Massachusetts Institute of Technology.
Schmitt, Otto H., Professor of Zoology, University of Minnesota.
Scholander, P. F., Swarthmore College.
Scott, Allan C., Associate Professor of Biology, Union College.
Scott, Sister Florence Marie, Professor of Biology, Seton Hill College.
Scott, George T., Associate Professor of Zoology, Oberlin College.
Seaman, Gerald R., Research Fellow, Fordham University.
Shanes, A. M., Associate Professor of Physiology, Georgetown University School of Medicine.
Sichel, F. J. M., Professor of Physiology, University of Vermont College of Medicine.
Slifer, Eleanor H., Assistant Professor of Zoology, State University of Iowa.
Speidel, Carl C., Professor of Anatomy, University of Virginia.
Steinbach, H. B., Professor of Zoology, University of Minnesota.
Stokey, Alma G., Professor of Botany *Emeritus,* Mount Holyoke College.

Stoudt, H. N., Instructor in Biology, Temple University.
Straus, William L., Associate Professor of Anatomy, Johns Hopkins University School of Medicine.
Stunkard, Horace H., Professor of Biology, New York University.
Suckling, Eustace E., Instructor, Long Island Medical College.
Szent-Gyorgyi, A. E., Institute for Muscle Research.
Taylor, Lauriston S., Chief Biophysics Branch, Atomic Energy Commission.
Taylor, Wm. Randolph, Professor of Botany, University of Michigan.
Terry, Robert L., Assistant Professor of Biology, Union College.
TeWinkel, Lois E., Associate Professor of Zoology, Smith College.
Ting, Te-pang, Research Associate, Amherst College.
Tracy, Henry C., Professor of Anatomy, University of Kansas.
Trinkaus, J. P., Instructor in Zoology, Osborn Zoological Laboratory.
Truant, A. P., Assistant Professor of Pharmacology, George Washington University School of Medicine.
Tyler, Albert, Associate Professor of Embryology, California Institute of Technology.
Varga, Laszle, Institute for Muscle Research.
Wald, George, Professor of Biology, Harvard University.
Warburg, Otto, Visiting Lecturer, Kaiser Wilhelm Institut for Physiology, Berlin.
Warner, Robert C., Assistant Professor of Chemistry, New York University College of Medicine.
Wenrich, D. H., Professor of Zoology, University of Pennsylvania.
Whiting, P. W., Professor of Zoology, University of Pennsylvania.
Wichterman, Ralph, Associate Professor of Biology, Temple University.
Wilber, Charles G., Director Biological Laboratories, Saint Louis University.
Willier, B. H., Director, Biological Laboratories, Johns Hopkins University.
Wilson, Walter L., Research Associate, University of Pennsylvania.
Wood, Richard D., Instructor in Botany, Rhode Island State College.
Woodward, Alvalyn E., Assistant Professor of Zoology, University of Michigan.
Woodward, Arthur A., Assistant Professor of Zoology, Brown University.
Wrinch, Dorothy, Lecturer in Physics, Smith College.
Wulff, Verner J., Assistant Professor of Zoology, University of Illinois.
Zalokar, Marko, Research Fellow, California Institute of Technology.
Zeuthen, Erik, Lecturer, University of Copenhagen.

Beginning Investigators, 1949

Bacon, Charles R. T., Student, University of Pennsylvania.
Battley, Edwin H., Student, Harvard University.
Batty, Thomas V., Graduate Student, University of Kansas.
Bauer, Mark H., Graduate Student, Princeton University.
Boyle, E. Marie, Science Teacher, The Baldwin School.
Carlson, Francis D., Instructor, Johns Hopkins University.
Cookson, Brian A., Cancer Research Fellow, University of Pennsylvania.
Essner, Edward S., Assistant Instructor, University of Pennsylvania.
Evans, Jeanne F., Student, University of Pennsylvania.
Flood, Veronica M., Junior Biochemist, Argonne National Laboratory.
Gagnon, Andre, University of Pennsylvania.
Gasvoda, Betty, Junior Biochemist, Argonne National Laboratory.
Gennaro, Joseph F., University of Pittsburgh.
Halaban, Atida, Graduate Student, University of Pennsylvania.
Harding, Clifford V., University of Pennsylvania.
Harding, Drusilla, University of Pennsylvania.
Hay, Elizabeth D., Student, Johns Hopkins Medical School.
Himmelfarb, Sylvia, University of Maryland Medical School.
Hirschfield, Henry I., Research Associate, University of Missouri.
Hoffman, Joseph F., Graduate Student, Princeton University.

Honegger, Carol M., Instructor, Temple University.
Hopkins, Amos L., Graduate Student, University of Pennsylvania.
Houlihan, Robert K., Graduate Assistant, Boston College.
Jacobs, Bertrand R., Student, University of Pennsylvania School of Medicine.
Kaplan, Ann Esther, Mount Holyoke College.
Kayhart, Marion, University of Pennsylvania.
Kelly, Elizabeth M., Graduate Assistant, University of Delaware.
Kelly, John W., Graduate Student, University of Pennsylvania.
Kirschner, Leonard B., University of Wisconsin.
Love, Warner E., University of Pennsylvania.
Malan, Martha, University of Pennsylvania.
McCay, Paul B., University of Oklahoma.
McCulloch, David, Teaching Fellow, Massachusetts Institute of Technology.
McKeehan, Morris S., Zoology Assistant, University of Chicago.
Miller, Tilford D., Amherst University.
Mitchell, Ruth, Columbia University.
Moskovic, Samuel, Teaching Fellow, New York University.
Moulton, James Malcolm, Teaching Fellow, Harvard University.
O'Malley, Benedict B., Graduate Student, Fordham University.
Padykula, Helen A., Instructor in Zoology, Wellesley College.
Pfister, Ronald R., Columbia Medical School.
Proctor, Nathaniel K., Graduate Student, University of Pennsylvania.
Rawley, June, Instructor, Kent State University.
Saltz, Myron, Student, Amherst College.
Schreibman, Irvin, University of Pennsylvania.
Sclufer, Evelyn, Graduate Student, Bryn Mawr College.
Seki, Sada L., Graduate Assistant, Mount Holyoke College.
Stieglitz, Alice A., Student, University of Pennsylvania.
Strittmatter, Cornelius F., Harvard University Medical School.
Tietze, Frank, Research Fellow, Northwestern University.
Vogel, Martin L., Amherst College.
Wilson, Thomas H., Instructor in Physiology, University of Pennsylvania.

Research Assistants, 1949

Abramsky, Tess, Georgetown University.
Andrews, J. R., United States Public Health Service.
Benson, Eleanore, University of Missouri.
Bernatowicz, Albert J., University of Michigan.
Blumenthal, Gertrude, University of Missouri.
Brumm, Anne F., New York University.
Bullock, Jane A., University of Oklahoma.
Burke, Dean, National Cancer Institute.
Curtis, Paul, Oberlin College.
Davidson, Margaret E., McGill University.
Fass, Jerome S., Rockefeller Institute.
Foley, Mary T., Yale University.
Friedler, Gladys, University of Pennsylvania.
Fromberg, Vivian, Woman's Medical College of Pennsylvania.
Garnic, Justine, Northwestern University.
Goldring, Roberta, Vassar College.
Gourevitch, Harry G., University of Chicago.
Grim, Madelon R., Amherst College.
Holland, Bert, University of Chicago.
Howard, Robert S., University of Miami.
Hsu, Dorothy L., University of Pennsylvania.
Jaffee, Oscar, Graduate School of Arts and Science, New York University.
Kun, Ernest, University of Chicago.

Lee, Lois E., Southwest Missouri State College.
Leonard, Lawrence, Haverford College.
Litt, Mortimer, University of Rochester Medical School.
Loeffler, Robert J., University of Wisconsin.
Lynn, Francis, Stanford University.
Miller, Faith S., Emory University.
Mitchell, Constance J., University of Delaware.
Moos, Carl, Massachusetts Institute of Technology.
Orski, Barbara M., Harvard Medical School.
Rosenbluth, Raja, Columbia University.
Sandeen, Muriel I., Northwestern University.
Slattery, Leo F., University of Chicago.
Stapleton, R. H., United States Public Health Service.
Stout, Carolyn M., University of Pennsylvania.
Talpey, William B., Washington University.
Walters, C. Patricia, Eli Lilly and Company.
Webb, H. Marguerite, Northwestern University.
Weber, Patricia, St. Louis University.
West, Alice, Radcliffe College.
Wilson, Marie, Northwestern University.
Winblad, James N., University of Kansas.

Library Readers, 1949

Adler, Francis H., Professor of Ophthalmology, University of Pennsylvania.
Bartlett, James H., Professor of Physics, University of Illinois.
Bloch, Robert, Research Associate, Yale University.
Bodian, David, Associate Professor in Epidemiology, Johns Hopkins University.
Cantoni, G. L., New York University.
Case, James F., Graduate Student, Johns Hopkins University.
Clendenning, Kenneth A., Associate Research Biologist, National Research Council, Canada.
Collier, Albert, Marine Biologist, Gulf Oil Corporation.
Conklin, Ruth E., Professor of Physiology, Vassar College.
Csaky, Tihamer Z., Research Associate, Duke Hospital.
Dempsey, Edward W., Harvard Medical School.
Edsall, Geoffrey, Professor of Bacteriology, Boston University School of Medicine.
Eichel, Bertram, Assistant Professor, Rutgers University.
Eichel, Herbert J., Teaching Assistant in Physiology, Rutgers University.
Flagler, Elizabeth A., Princeton University.
Freund, Jules, Chief, Division of Applied Immunology, Public Health Institute of New York City.
Gabriel, Mordecai L., Assistant Professor of Biology, Brooklyn College.
Gates, R. R., Research Fellow, Harvard University.
Grant, Maleleine P., Member of Science Faculty, Sarah Lawrence College.
Gudernatsch, Frederick, 41 Fifth Avenue, New York 3, New York.
Gurewich, Vladimir, Assistant Visiting Physician, Bellevue Hospital.
Hausler, Hans F., Head of Pharmacological Department, University of Graz.
Hobson, Lawrence B., Associate Medical Director, E. R. Squibb and Sons.
Kabat, Elvin A., Associate Professor of Bacteriology, Neurological Institute.
Karush, Fred, Fellow, Sloan-Kettering Institute for Cancer Research.
Keller, Rudolph, Director of Research, Madison Foundation.
Keosian, John, Professor of Biology, Newark College, Rutgers University.
Kindred, James E., Professor of Anatomy, University of Virginia.
Kozam, George, Instructor in Anatomy, New York University.
Krasnow, Frances, Head, Department of Research, The Guggenheim Dental Foundation.
Kuff, Edward L., Instructor in Anatomy, Washington University School of Medicine.
Leikind, Morris C., Head of Biology and Medical Unit, Library of Congress.
Ling, Gilbert N., Postdoctorate Fellow, University of Chicago.

Loewi, Otto, Research Professor, New York University College of Medicine.
Mavor, James W., Professor of Biology *Emeritus*, Union College.
McDonald, Sister Elizabeth Seton, Professor of Biology, College of Mount St. Joseph.
McLean, Dorothy J., Instructor, Vassar College.
Meyerhof, Otto, Research Professor of Biochemistry, University of Pennsylvania.
Morrison, David, Flight Safety Foundation, Inc.
Nadeau, L. V., Graduate Student, Dominican House of Studies.
Perkins, John F., Jr., Assistant Professor, University of Chicago.
Quastel, J. H., Professor of Biochemistry, McGill University.
Renn, Charles E., Associate Professor of Sanitary Engineering, Johns Hopkins University.
Root, Raymond W., Associate Professor of Biology, College of the City of New York.
Roys, Chester, Graduate Student, University of Iowa.
Shwartzman, Gregory, Bacteriologist, Mount Sinai Hospital.
Smith, Mary F., University of Oklahoma.
Sulkin, S. Edward, Professor of Bacteriology, Southwestern Medical College.
Sutro, Peter J., Graduate Student, Harvard University.
Tannenbaum, Stuart, Graduate Student, Columbia University.
Therman, P. O., Institute of Pennsylvania Hospital.
Thomson, Betty F., Assistant Professor of Botany, Connecticut College.
Wainio, Walter W., Assistant Research Specialist, Rutgers University.
Wittenberg, Jonathan, Graduate Student, Columbia University.
Zorzoli, Anita, Washington University.

Students, 1949

BOTANY

Barker, Frances M., Assistant in Bacteriology, Wellesley College.
Benoit, Richard J., Graduate Student, Yale University.
Bubieniec, Ernest J., Student, Harvard College.
Chismore, Joyce M., Student, Yale University.
Conover, John T., Student, University of Michigan.
Hesse, Alice van Voorst, Student, 190 Riverside Drive, New York City 24.
King, John W., Professor, Morgan State College.
Lane, Susan A., Assistant in Bacteriology, University of Vermont.
Lewin, Ralph A., Graduate Student, Yale University.
Loeffler, Robert J., University of Wisconsin.
Putala, Eugene C., Student, University of Massachusetts.
Rieger, Cecilia A., Hofstra College.
Smith, Margaret J., Vassar College.
Stonier, Tom T., Student, Drew University.
Straus, Jacob, Student, College of the City of New York.

EMBRYOLOGY

Absolon, Karel B., June Brown Memorial Fellow in Pathology, Yale University.
Aldrich, Fred A., 49 Boonton Avenue, Butler, New Jersey.
Blauch, Bertina M., Student, University of Pennsylvania.
Blum, Sister M. Georgiana, Student, Marquette University.
Brooks, Harold K., Graduate Student, Harvard University.
Case, James F., Graduate Student, Johns Hopkins University.
Chiquoine, A. Duncan, Cornell University.
Cohen, Adolph I., Graduate Student, Columbia University.
Davidheiser, Roger H., Student, University of New Hampshire.
Finnegan, Cyril V., Jr., Research Assistant, University of Notre Dame.
Gage, Margaret E., Student, DePauw University.
Guyselman, J. Bruce, Graduate Assistant, Northwestern University.
Heacock, Ruth E., Student, Oberlin College.

Kitts, David B., University of Pennsylvania.
Kuta, Virginia A., Graduate Assistant, DePaul University.
Lawlor, Rev. George F., Instructor, Boston College.
McKeehan, Morris S., Student, University of Chicago.
Mirand, Edwin A., Teaching Fellow, Syracuse University.
Montreuil, Paul, McGill University.
Nerad, Josephine J., Graduate Assistant, DePaul University.
Oliver, Sidney W., Instructor of Biology, University of South Carolina.
Orsi, Ernest V., Student, Fordham University.
Prescott, David M., Student, Wesleyan University.
Quen, Jacques M., Graduate Assistant, Brown University.
Rogers, Kay T., Research Associate, Harvard University.
Shaw, Edward I., Graduate Assistant in Zoology, University of Missouri.
Smolker, Robert E., Boston University.
Townes, Philip L., Graduate Student, University of Rochester.
Turner, Ruth D., Research Assistant, Harvard University.
Vinson, Cordelia A., Graduate Assistant, University of North Carolina.
Vincent, Walter S., Jr., Graduate Student, University of Pennsylvania.

PHYSIOLOGY

Avery, Mary E., Student, Johns Hopkins School of Medicine.
Barton, Jay, II, Graduate and Research Assistant, University of Missouri.
Cambel, Perihan, Fellow in Cancer Research, Washington University.
Dorais, Jeanne M. C., Graduate Assistant, Vassar College.
Durell, Jack, Harvard University.
Gosselin, M. Lorraine, Technician, Rockefeller Institute for Medical Research.
Jacob, Miriam I., Technician, Rockefeller Institute for Medical Research.
Johnson, Phyllis E., Graduate Assistant in Biochemistry, Mount Holyoke College.
Kahn, Arthur J., Research Associate, New York University.
Klein, Richard, Fellow National Cancer Institute, University of Rochester.
Kline, Raymond F., Graduate Student, University of Maryland Medical School.
Kolin, Alexander, Assistant Professor of Physics, University of Chicago.
Lesse, Henry, Student, Jefferson Medical College.
McIntyre, Patricia A., Student, Johns Hopkins University School of Medicine.
Michel, Burlyn E., Student, University of Chicago.
Montague, Laura J., Student, Johns Hopkins Medical School.
Passano, Leonard M., Graduate Student, Yale University.
Schiffman, Gerald, Student, New York University College of Medicine.
Schwartz, Martin, Graduate Student, Clark University.
Sheng, Ginger, Student, Columbia University.
Smith, Jay A., Assistant Professor of Physiology, Chicago Medical School.
Snyder, Robert A., University of Western Ontario.
Stadler, David R., Graduate Student, Princeton University.
Swenson, Paul A., Stanford University.
Thompson, Alan M., Student, Iowa State College.
Thornburg, Wayne, Student, University of Illinois.
Weimar, Virginia L., University of Pennsylvania.

INVERTEBRATE ZOOLOGY

Amberson, Barbara D., Pennsylvania State College.
Amos, Patsy D., Student, Oberlin College.
Anderson, Betsy J., Student, Drury College.
Anderson, Victor Elving, Student, University of Minnesota.
Auerbach, Earl, Graduate Student, Northwestern University.
Barber, Saul B., Graduate Student, Yale University.
Bellisario, Joseph, Laboratory Instructor, Washington and Jefferson College.

Birch, Robert Lee, Instructor in Zoology, West Virginia University.
Bouwman, Frederick L., Jr., Graduate Assistant, Michigan State College.
Bullock, Jane Ann, Graduate Student, University of Oklahoma.
Burnett, Frances L., Cornell University.
Chaet, Alfred B., Student, University of Massachusetts.
Currier, Joanne E., Graduate Assistant in Zoology, Vassar College.
D'Ambrosio, Gloria, Student, Northwestern University.
Davis, Earle A., Jr., Graduate Student, University of Illinois.
Dawson, William A., Student, Swarthmore College.
Donnelly, Orville W., Graduate Assistant, Williams College.
Fienemann, Barbara J., Teaching Fellow, Smith College.
Fingerman, Milton, Graduate Assistant, Northwestern University.
Foster, Richard W., Assistant Curator of Mollusks, Harvard College.
Frost, Robert C., Graduate Student, Rice Institute.
Gerold, Nicolas J., Jr., Assistant, Cornell University.
Getman, Herbert C., Graduate Student, Brown University.
Gilmartin, Rosemary T., Graduate Student, Yale University.
Godfrey, Robert R., Graduate Assistant, New York University.
Goldstein, Lester, Graduate Student, University of Pennsylvania.
Grant, William C., Dartmouth College.
Grun, John, Student, Brooklyn College.
Hankins, Robert M., Research Assistant, University of Kansas.
Hansen, Bruce W., Student, Monmouth College.
Holle, Paul A., Graduate Student, University of Notre Dame.
James, Carolyn L., Vassar College.
Jones, Elizabeth J., Undergraduate, Oberlin College.
Kallen, Eugene J., Student, Columbia University.
Leonard, Barbara H., Instructor, Smith College.
Mabel, Judith B., Student, Goucher College.
Most, Sylvia N., Graduate Student, University of Pennsylvania.
Nanney, David, Research Fellow, Indiana University.
Patten, John A., New York University.
Peck, Harry D., Student, Wesleyan University.
Pulley, Thomas E., Assistant Professor, University of Houston.
Rieck, Alvin F., Marquette University.
Said, Rushdi, Student, Harvard University.
Schneiderman, Howard A., Fellow in Biology, Harvard University.
Sedar, Albert W., Student, State University of Iowa.
Sengbusch, Howard G., Graduate Assistant, New York University.
Sindermann, Carl J., University of Massachusetts.
Sippel, Theodore O., Laboratory Assistant in Zoology, Yale University.
Spiegel, Melvin, Graduate Student, University of Rochester.
Stafford, Nancy J., Teaching Fellow, Tufts College.
Telfer, William H., Graduate Student, Harvard University.
Tinker, Milton E., Teaching Assistant, University of Minnesota.
Weyl, Hilda, Student, Hunter College.
Williams, Austin B., Assistant Instructor, Kansas University.
Wilson, Katherine, Graduate Assistant, Washington University.

5. The Lalor Fellows, 1949

Albert W. Frenkel, University of Minnesota.
C. Albert Kind, University of Connecticut.
Albert I. Lansing, Washington University School of Medicine.
Jerre L. Noland, University of Wisconsin.
Arthur A. Woodward, Brown University.
Verner J. Wulff, University of Illinois.

6. The Atomic Energy Commission Fellows

Richard Abrams, University of Chicago.
Felix Friedberg, Howard University.
Marion Kayhart, University of Pennsylvania.
Jay S. Roth, Rutgers University.
Frank H. Rudenberg, Harvard University.

7. Tabular View of Attendance, 1945–1949

	1945	*1946*	*1947*	*1948*	*1949*
Investigators—Total	212	267	299	326	344
Independent	138	175	182	183	193
Under instruction	10	29	36	42	52
Library readers	38	38	36	50	55
Research Assistants	26	25	45		
Students—Total	96	122	131	123	128
Zoology	55	57	55	54	55
Embryology	23	30	33	29	31
Physiology	13	26	26	25	27
Botany	5	9	17	15	15
Total Attendance	308	389	430	449	472
Less persons registered as both students and investigators			2	6	2
			428	*443*	*470*
Institutions Represented—Total	*124*	*141*	*148*	*158*	*155*
By investigators	100	102	114	117	114
By students	49	56	56	68	68
Schools and Academies Represented					
By investigators	2	2	1	1	1
By students	2		1		
Foreign Institutions Represented					
By investigators	1	7	7	8	6
By students		5	3	4	3

8. Subscribing and Cooperating Institutions, 1949

Subscribing Institutions

Argonne National Laboratory
Boston University
George Washington University Medical School
Georgetown University Medical School
Indiana University
Institute for Cancer Research, Lankenau Hospital
Institute of Pennsylvania Hospital
Long Island Medical College
Marquette University
Morgan State College
National Research Council
National Research Council of Canada
Northwestern University
Ohio State University, Graduate School
Public Health Research Institute of New York City
Radcliffe College
Russell Sage College
Rutgers University
St. Louis University
Southwestern Medical College
Tulane University School of Medicine
U. S. Public Health Service
University of New Hampshire
University of Oklahoma
University of Pittsburgh
University of South Dakota
University of Wisconsin
Wellesley College

Cooperating Institutions

Amherst College
Brooklyn College
Brown University
Bryn Mawr College
Catholic University of America
College of Mt. St. Joseph-on-the-Ohio
College of Physicians and Surgeons
Columbia University
Cornell University
Duke University
Fordham University
Goucher College
Harvard University
Harvard University Medical School
Hunter College
Institute for Muscle Research
Johns Hopkins University
Johns Hopkins University Medical School
Eli Lilly and Company
Madison Foundation
Massachusetts Institute of Technology
Mount Holyoke College
Mount Sinai Hospital
National Cancer Institute
New York University College of Medicine
New York University, Heights
New York University, Washington Square College
North Carolina State College of Agriculture and Engineering
Oberlin College
Princeton University
Rockefeller Institute for Medical Research
Sloan-Kettering Institute
Smith College
State University of Iowa
Temple University
Tufts College Medical School
Union College
University of Chicago
University of Delaware
University of Illinois
University of Kansas
University of Maryland School of Medicine
University of Massachusetts
University of Michigan
University of Minnesota
University of Missouri
University of Pennsylvania Medical School
University of Pennsylvania
University of Vermont Medical School
University of Virginia
Vassar College
Washington University
Wesleyan University
Western Reserve University
Yale University

9. Evening Lectures

The Friday Evening Lectures, 1949

Friday, July 1
Dr. Albert Tyler .... "Serological Aspects of Fertilization."
Friday, July 8
Dr. Hans Gaffron .... "Problems in Photosynthesis."
Friday, July 15
Dr. J. H. Quastel .... "Soil as a Medium for Biochemical Investigations."
Friday, July 22
Dr. Erik Zeuthen .... "Recent Developments in the Cartesian Diver Technique, and Its Application to the Study of Respiration During Mitosis."
Friday, July 29
Dr. T. M. Sonneborn .... "Environment, Cytoplasm, and Genes in the Control of Heredity in Paramoecium."
Friday, August 5
Dr. J. S. Fruton .... "The Metabolism of Peptides."
Friday, August 12
Dr. F. A. Brown, Jr. .... "The Mechanism of Color Changes in Crustacea."
Friday, August 19
Dr. M. E. Krahl .... "Metabolic Activities of the Arbacia Egg."
Friday, August 26
Dr. S. Gross .... "Experiments in Marine Fish Culture."

Other Lectures

Wednesday, June 15
Dr. Gunnar Thorson .... "Reproductive and Larval Ecology of Marine Bottom Invertebrates."
Monday, July 18
Dr. Perihan Cambel .... "Problems of Modern Turkey."
Wednesday, July 27
L. V. Foster .... "Microscope Optics."
Wednesday, August 24
Dr. Howard A. Meyerhoff ....
Tuesday, August 30
Dr. Michael Graham .... "Recent Marine Research in the Arctic."

10. Seminars, 1949

July 12
Anna R. Whiting .... "Evidence that Response to Fractionation of X-ray Dose in Habrobracon Eggs is Cytoplasmic."
H. H. Plough, Madelon R. Grimm and M. L. Vogel .... "Minus and Plus Mutations in Biochemical Requirements in *Salmonella typhimurium*."
P. W. Whiting and Marion E. Kayhart .... "X-ray Mutations and Fecundity of Mormoniella."
July 19
Albert Frenkel .... "The Effect of Ultra-Violet Light (2537 Å) on the Catalase Activity and on Photosynthesis of *Chlorella pyrenoidosa*."
Ernest Kun .... "Biochemical Properties of Succinoxidase from *Salmonella aertrycke*."
Alfred Bliss .... "Reversible Enzymic Reduction of Retinene to Vitamin A."
David Nachmansohn, S. Hestrin and H. Voripaieff .... "An Enzymatic Product with Acetylcholine-like Activity, Derived from Brain Extracts."
July 26
Ethel Browne Harvey .... "Growth and Metamorphosis of the Pluteus of *Arbacia punctulata*."
James A. Miller .... "Effects of Temperature upon Survival of Newborn Guinea Pigs Subjected to Anoxia."
Alfred Marshak .... "Evidence for Activity of Desoxy-ribonuclease in Nuclear Fusion and Mitosis by the Use of d-Usnic Acid."
Carl C. Speidel .... "Motion Pictures Showing the Reactions of Cells in Frog Tadpoles to Implants of Tantalum."
August 2
Andrew G. Szent-Gyorgyi .... "Investigations on Muscle Fibers."

E. L. Chambers and W. E. White	"The Accumulation of Orthophosphate and its Relation to Adenosine Triphosphate in the Fertilized Echinoderm Egg."
Abel Lajtha	"Energy Rich Phosphate in Nucleic Acids."
Elemer Mihalyi	"On the Structure of Fibrin Clots."
Brian A. Cookson	"Some Methods of Producing Traveling Contraction Nodes in Adult Frog Skeletal Muscle Fibers."
August 9	
F. R. Hunter	"An Analysis of the Photoelectric Method of Measuring Permeability."
M. H. Jacobs, Carolyn M. Stout, Marion W. LeFevre and W. E. Love	"How Simple Are the So-called Simple Hemolysins?"
Lois H. Love	"Hemolytic Action of Anionic Detergents."
A. K. Parpart and J. W. Green	"Potassium and Sodium Exchange in Rabbit Erythrocytes Treated with Butyl Alcohol."
August 16	
Dorothy Wrinch	"Structure of Insulin."
J. M. Moulton	"The Development of Menidia-Fundulus Hybrids."
Ezra Allen	"Analysis of Degeneration in Primordial Germ Cells and a Hitherto Undescribed Germ Cell in Albino Male Rats."
C. G. Wilber	"Fat Metabolism in Arctic Fish as Compared with Temperate Form."

II. Members of the Corporation, 1949

1. Life Members

Beckwith, Dr. Cora J., Vassar College, Poughkeepsie, New York.
Billings, Mr. R. C., 66 Franklin Street, Boston, Massachusetts.
Calvert, Dr. Philip P., University of Pennsylvania, Philadelphia, Pennsylvania.
Cole, Dr. Leon J., College of Agriculture, Madison, Wisconsin.
Conklin, Prof. Edwin G., Princeton University, Princeton, New Jersey.
Cowdry, Dr. E. V., Washington University, St. Louis, Missouri.
Jackson, Mr. Chas. C., 24 Congress Street, Boston, Massachusetts.
Jackson, Miss M. C., 88 Marlboro Street, Boston, Massachusetts.
King, Mr. Chas. A.
Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Maryland.
Means, Dr. J. H., 15 Chestnut Street, Boston, Massachusetts.
Moore, Dr. George T., Missouri Botanical Gardens, St. Louis, Missouri.
Moore, Dr. J. Percy, University of Pennsylvania, Philadelphia, Pa.
Morgan, Mrs. T. H., Pasadena, California.
Noyes, Miss Eva J.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.
Scott, Dr. Ernest L., Columbia University, New York City, New York.
Sears, Dr. Henry F., 86 Beacon Street, Boston, Massachusetts.
Shedd, Mr. E. A.

Strong, Dr. O. S., Columbia University, New York City, New York.
Waite, Prof. F. C., 144 Locust Street, Dover, New Hampshire.
Wallace, Louise B., 359 Lytton Avenue, Palo Alto, California.

2. Regular Members

Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Massachusetts.
Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
Adolph, Dr. Edward F., University of Rochester Medical School, Rochester, New York.
Albaum, Dr. Harry G., Biology Dept., Brooklyn College, Brooklyn, N. Y.
Albert, Dr. Alexander, Mayo Clinic, Rochester, Minnesota.
Allee, Dr. W. C., The University of Chicago, Chicago, Illinois.
Amberson, Dr. William R., Department of Physiology, University of Maryland, School of Medicine, Baltimore, Md.
Anderson, Dr. Rubert S., Department of Physiology, University of South Dakota, Vermillion, South Dakota.
Anderson, Dr. T. F., University of Pennsylvania, Philadelphia, Pennsylvania.
Angerer, Prof. C. A., Department of Physiology, Ohio State College, Columbus, Ohio.
Armstrong, Dr. Philip B., College of Medicine, Syracuse University, Syracuse, New York.
Austin, Dr. Mary L., Wellesley College, Wellesley, Massachusetts.
Baitsell, Dr. George A., Yale University, New Haven, Connecticut.
Baker, Dr. H. B., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.
Ball, Dr. Eric G., Department of Biological Chemistry, Harvard University Medical School, Boston, Massachusetts.
Ballard, Dr. William W., Dartmouth College, Hanover, New Hampshire.
Bard, Prof. Philip, Johns Hopkins Medical School, Baltimore, Maryland.
Barron, Dr. E. S. Guzman, Department of Medicine, The University of Chicago, Chicago, Illinois.
Barth, Dr. L. G., Department of Zoology, Columbia University, New York City, New York.
Bartlett, Dr. James H., Department of Physics, University of Illinois, Urbana, Illinois.
Beadle, Dr. G. W., California Institute of Technology, Pasadena, California.
Beams, Dr. Harold W., Department of Zoology, State University of Iowa, Iowa City, Iowa.
Beck, Dr. L. V., 5609 Roosevelt St., Bethesda, Maryland.
Beers, C. D., University of North Carolina, Chapel Hill, North Carolina.
Behre, Dr. Elinor H., Louisiana State University, Baton Rouge, Louisiana.
Bertholf, Dr. Lloyd M., College of the Pacific, Stockton, California.
Bevelander, Dr. Gerrit, New York University School of Medicine, New York City, New York.
Bigelow, Dr. H. B., Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts.

Bigelow, Prof. R. P., Massachusetts Institute of Technology, Cambridge, Massachusetts.

Bissonnette, Dr. T. Hume, Trinity College, Hartford, Connecticut.

Blanchard, Prof. K. C., Johns Hopkins Medical School, Baltimore, Maryland.

Bliss, Dr. Alfred F., Department of Physiology, Tufts College Medical School, Boston, Mass.

Blum, Dr. Harold F., Department of Biology, Princeton University, Princeton, New Jersey.

Bodian, Dr. David, Department of Epidemiology, Johns Hopkins University, Baltimore, Maryland.

Bodine, Dr. J. H., Department of Zoology, State University of Iowa, Iowa City, Iowa.

Boell, Dr. Edgar J., Yale University, New Haven, Connecticut.

Bonner, Dr. John T., Department of Biology, Princeton University, Princeton, New Jersey.

Boring, Dr. Alice M., Yenching University, Peiping, China.

Bradley, Prof. Harold C., 2639 Durant Avenue, Berkeley 4, California.

Brodie, Mr. Donald M., 522 Fifth Avenue, New York City, New York.

Bronfenbrenner, Dr. Jacques J., Department of Bacteriology, Washington University Medical School, St. Louis, Missouri.

Bronk, Dr. Detlev W., Johns Hopkins University, Baltimore, Maryland.

Brooks, Dr. Matilda M., University of California, Department of Zoology, Berkeley, California.

Brown, Dr. Dugald E. S., Department of Zoology, University of Michigan, Ann Arbor, Michigan.

Brown, Dr. Frank A., Jr., Department of Zoology, Northwestern University, Evanston, Illinois.

Brownell, Dr. Katherine A., Ohio State University, Columbus, Ohio.

Buck, Dr. John B., Industrial Hygiene Research Lab., National Institute of Health, Bethesda, Maryland.

Buckingham, Miss Edith N., Sudbury, Massachusetts.

Budington, Prof. R. A., Winter Park, Box 944, Florida.

Bullington, Dr. W. E., Randolph-Macon College, Ashland, Virginia.

Bullock, Dr. T. H., University of California, Los Angeles 24, California.

Burbanck, Dr. William D., Department of Biology, Drury College, Springfield, Missouri.

Burkenroad, Dr. M. D., Bogue Sound Road, Newport, North Carolina.

Butler, Dr. E. G., Princeton University, Princeton, N. J.

Cameron, Dr. J. A., Baylor College of Dentistry, Dallas, Texas.

Cannan, Prof. R. K., New York University College of Medicine, New York City, New York.

Carlson, Prof. A. J., Department of Physiology, The University of Chicago, Chicago, Illinois.

Carothers, Dr. E. Eleanor, 134 Avenue C. East, Kingman, Kansas.

Carpenter, Dr. Russell L., Tufts College, Tufts College, Massachusetts.

Carver, Prof. Gail L., Mercer University, Macon, Georgia.

Cattell, Dr. McKeen, Cornell University Medical College, New York City, New York.

Cattell, Mr. Ware, Cosmos Club, Washington, D. C.
Chambers, Dr. Robert, Woods Hole, Massachusetts.
Chase, Dr. Aurin M., Princeton University, Princeton, New Jersey.
Cheney, Dr. Ralph H., Biology Department, Brooklyn College, Brooklyn 10, New York.
Child, Prof. C. M., Jordan Hall, Stanford University, California.
Churney, Dr. Leon, Dept. of Physiology, Louisiana State University School of Medicine, New Orleans 13, Louisiana.
Claff, Mr. C. Lloyd, 31 West Street, Randolph, Massachusetts.
Clark, Dr. A. M., Department of Biology, University of Delaware, Newark, Delaware.
Clark, Prof. E. R., Wistar Institute, Woodland Avenue and 36th Street, Philadelphia 4, Pennsylvania.
Clark, Dr. Leonard B., Department of Biology, Union College, Schenectady, New York.
Clarke, Dr. G. L., Department of Biology, Harvard University, Cambridge 38, Mass.
Cleland, Prof. Ralph E., Indiana University, Bloomington, Indiana.
Clement, Dr. A. C., Department of Biology, Emory University, Emory, Georgia.
Clowes, Dr. G. H. A., Eli Lilly and Company, Indianapolis, Indiana.
Coe, Prof. W. R., Scripps Institute of Oceanography, La Jolla, California.
Cohn, Dr. Edwin J., 183 Brattle Street, Cambridge, Massachusetts.
Cole, Dr. Elbert C., Department of Biology, Williams College, Williamstown, Massachusetts.
Cole, Dr. Kenneth S., Naval Medical Research Institute, Bethesda 14, Maryland.
Collett, Dr. Mary E., Western Reserve University, Mather College, Cleveland, Ohio.
Colton, Prof. H. S., Box 601, Flagstaff, Arizona.
Colwin, Dr. Arthur L., Queens College, Flushing, Long Island, New York.
Colwin, Dr. Laura N. H., Department of Biology, Queens College, Flushing, New York.
Cooper, Dr. Kenneth W., Department of Biology, Princeton University, Princeton, New Jersey.
Copeland, Dr. D. E., Department of Zoology, Brown University, Providence, Rhode Island.
Copeland, Prof. Manton, Bowdoin College, Brunswick, Maine.
Cornman, Dr. Ivor, George Washington University, Warwick Memorial Clinic, Washington 5, D. C.
Costello, Dr. Donald P., Department of Zoology, University of North Carolina, Chapel Hill, North Carolina.
Costello, Dr. Helen Miller, Department of Zoology, University of North Carolina, Chapel Hill, North Carolina.
Crampton, Prof. H. E., American Museum of Natural History, New York City, New York.
Crane, John O., Woods Hole, Massachusetts.
Crane, Mrs. W. Murray, Woods Hole, Massachusetts.
Croasdale, Hannah T., Dartmouth College, Hanover, New Hampshire.

Crouse, Dr. Helen V., Goucher College, Baltimore, Maryland.
Crowell, Dr. P. S., Jr., Department of Zoology, University of Indiana, Bloomington, Indiana.
Curtis, Dr. Maynie R., 4811 John R. Street, Detroit, Michigan.
Curtis, Prof. W. C., University of Missouri, Columbia, Missouri.
Dan, Dr. Katsuma, Misaki Biological Station, Misaki, Japan.
Davis, Dr. Donald W., College of William and Mary, Williamsburg, Virginia.
Dawson, Dr. A. B., Harvard University, Cambridge, Massachusetts.
Dawson, Dr. J. A., The College of the City of New York, New York City, New York.
Dederer, Dr. Pauline H., Connecticut College, New London, Connecticut.
Demerec, Dr. M., Carnegie Institution of Washington, Cold Spring Harbor, Long Island, New York.
Diller, Dr. Irene C., Institute for Cancer Research, Philadelphia, Pennsylvania.
Diller, Dr. William P., 205 Fairhill Avenue, Glenside, Pennsylvania.
Dodds, Prof. G. S., Medical School, University of West Virginia, Morgantown, West Virginia.
Dolley, Prof. William L., University of Buffalo, Buffalo, New York.
Donaldson, Dr. John C., University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania.
Doty, Dr. Maxwell S., Northwestern University, Department of Botany, Evanston, Illinois.
Drinker, Dr. Cecil K., Box 502, Falmouth, Massachusetts.
DuBois, Dr. Eugene F., Cornell University Medical College, New York City, New York.
Duggar; Dr. Benjamin M., c/o Lederle Laboratories Inc., Pearl River, New York.
Dungay, Dr. Neil S., Carleton College, Northfield, Minnesota.
Duryee, Dr. William R., Carnegie Institute, 5241 Broad Branch Rd. N.W., Washington 15, D. C.
Evans, Dr. Titus C., State University of Iowa, Iowa City, Iowa.
Failla, Dr. G., College of Physicians and Surgeons, New York City, New York.
Fauré-Fremiet, Prof. Emmanuel, Collège de France, Paris, France.
Ferguson, Dr. F. P., Department of Physiology, University of Maryland Medical School, Baltimore, Maryland.
Ferguson, Dr. James K. W., Department of Pharmacology, University of Toronto, Ontario, Canada.
Figge, Dr. F. H. J., Lombard and Greene Streets, Baltimore, Maryland.
Fischer, Dr. Ernst, Baruch Centre of Physical Medicine, Medical College of Virginia, Richmond 19, Virginia.
Fisher, Dr. Jeanne M., Department of Biochemistry, University of Toronto, Toronto, Canada.
Fisher, Dr. Kenneth C., Department of Biology, University of Toronto, Toronto, Canada.
Forbes, Dr. Alexander, Biological Laboratories, Divinity Avenue, Cambridge, Massachusetts.
Frisch, Dr. John A., Canisius College, Buffalo, New York.

Furth, Dr. Jacob, 201 Delaware Avenue, Oak Ridge, Tennessee.
Gaffron, Dr. Hans, Department of Biochemistry, University of Chicago, Chicago 37, Illinois.
Galtsoff, Dr. Paul S., 420 Cumberland Avenue, Somerset, Chevy Chase, Maryland.
Garrey, Prof. W. E., Vanderbilt University Medical School, Nashville, Tennessee.
Gasser, Dr. Herbert, Director, Rockefeller Institute, New York City, New York.
Gates, Dr. Reginald R., Biological Laboratories, Harvard University, Cambridge, Massachusetts.
Geiser, Dr. S. W., Southern Methodist University, Dallas, Texas.
Gerard, Prof. R. W., The University of Chicago, Chicago, Illinois.
Gilman, Dr. L. C., Department of Zoology, University of Miami, Coral Gables, Florida.
Glaser, Prof. O. C., Amherst College, Amherst, Massachusetts.
Goldforb, Prof. A. J., College of the City of New York, New York City, New York.
Goodchild, Dr. Chauncey G., Missouri State College, Springfield, Missouri.
Gottschall, Dr. Gertrude Y., 315 East 68th Street, New York 21, New York.
Gould, Dr. H. N., Newcomb College, New Orleans 18, Louisiana.
Grand, Constantine G., Biology Department, Washington Square College, New York University, Washington Square, New York City, New York.
Grant, Dr. Madeleine P., Sarah Lawrence College, Bronxville, New York.
Gray, Prof. Irving E., Duke University, Durham, North Carolina.
Gregg, Dr. J. R., Department of Zoology, Columbia University, New York 27, New York.
Gregory, Dr. Louise H., 1160 Fifth Avenue, New York City, New York.
Grosch, Dr. Daniel S., Department of Zoology, North Carolina State College, Raleigh, North Carolina.
Grundfest, Dr. Harry, Columbia University College of Physicians and Surgeons, New York City, New York.
Gudernatsch, Dr. Frederick, 41 Fifth Avenue, New York 3, New York.
Guthrie, Dr. Mary J., University of Missouri, Columbia, Missouri.
Guyer, Prof. M. F., University of Wisconsin, Madison, Wisconsin.
Hague, Dr. Florence, Sweet Briar College, Sweet Briar, Virginia.
Hall, Prof. Frank G., Duke University, Durham, North Carolina.
Hamburger, Dr. Viktor, Department of Zoology, Washington University, St. Louis, Missouri.
Hamilton, Dr. Howard L., Iowa State College, Ames, Iowa.
Hance, Dr. Robert T., The Cincinnati Milling Machine Co., Cincinnati 9, Ohio.
Harman, Dr. Mary T., Kansas State Agricultural College, Manhattan, Kansas.
Harnly, Dr. Morris H., Washington Square College, New York University, New York City, New York.
Harrison, Prof. Ross G., Yale University, New Haven, Connecticut.
Hartline, Dr. H. Keffer, 215 Mergenthaler Hall, Johns Hopkins University, Baltimore, Maryland.
Hartman, Dr. Frank A., Hamilton Hall, Ohio State University, Columbus, Ohio.
Harvey, Dr. E. Newton, Guyot Hall, Princeton University, Princeton, New Jersey.

Harvey, Dr. Ethel Browne, 48 Cleveland Lane, Princeton, New Jersey.
Hauschka, Dr. T. S., Institute for Cancer Research, Philadelphia 30, Pennsylvania.
Hayashi, Dr. Teru, Columbia University, New York City, New York.
Hayden, Dr. Margaret A., Wellesley College, Wellesley, Massachusetts.
Hayes, Dr. Frederick R., Zoological Laboratory, Dalhousie University, Halifax, Nova Scotia.
Haywood, Dr. Charlotte, Mount Holyoke College, South Hadley, Massachusetts.
Heilbrunn, Dr. L. V., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.
Henley, Dr. Catherine, Department of Zoology, University of North Carolina, Chapel Hill, North Carolina.
Henshaw, Dr. Paul S., National Cancer Institute, Bethesda, Maryland.
Hess, Prof. Walter N., Hamilton College, Clinton, New York.
Hibbard, Dr. Hope, Department of Zoology, Oberlin College, Oberlin, Ohio.
Hill, Dr. Samuel E., 18 Collins Avenue, Troy, New York.
Hinrichs, Dr. Marie, University of Illinois Health Service, 807 South Wright, Champaign, Illinois.
Hisaw, Dr. F. L., Harvard University, Cambridge, Massachusetts.
Hoadley, Dr. Leigh, Biological Laboratories, Harvard University, Cambridge, Massachusetts.
Höber, Dr. Rudolf, University of Pennsylvania, Philadelphia, Pennsylvania.
Hodes, Dr. Robert, Tulane University School of Medicine, New Orleans, Lousiana.
Hodge, Dr. Charles, IV, Temple University, Department of Zoology, Philadelphia, Pennsylvania.
Hogue, Dr. Mary J., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
Hollaender, Dr. Alexander, P.O. Box W., Clinton Laboratories, Oak Ridge, Tennessee.
Hopkins, Dr. Dwight L., University of Illinois, Pier Branch—Navy Pier, Division of Biological Science, Chicago, Illinois.
Hopkins, Dr. Hoyt S., New York University, College of Dentistry, New York City, New York.
Hunter, Dr. Francis R., Department of Zoology, University of Oklahoma, Norman, Oklahoma.
Hutchens, Dr. John O., Department of Physiology, University of Chicago, Chicago 37, Illinois.
Hyman, Dr. Libbie H., American Museum of Natural History, New York City, New York.
Irving, Dr. Laurence, Swarthmore College, Department of Zoology, Swarthmore, Pennsylvania.
Iselin, Mr. Columbus O'D., Woods Hole, Massachusetts.
Jacobs, Prof. Merkel H., School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
Jenkins, Dr. George B., 1336 Parkwood Place, N.W., Washington, D. C.
Johlin, Dr. J. M., Vanderbilt University Medical School, Nashville, Tennessee.
Jones, Dr. E. Ruffin, University of Florida, Gainesville, Florida.

Kaan, Dr. Helen W., National Research Council, 2101 Constitution Avenue, Washington, D. C.

Kabat, Dr. E. A., Neurological Institute, College of Physicians and Surgeons, New York City, N. Y.

Kaufmann, Prof. B. P., Carnegie Institution, Cold Spring Harbor, Long Island, New York.

Kempton, Prof. Rudolf T., Vassar College, Poughkeepsie, New York.

Kille, Dr. Frank R., Carleton College, Northfield, Minnesota.

Kindred, Dr. J. E., University of Virginia, Charlottesville, Virginia.

King, Dr. Helen D., Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania.

King, Dr. Robert L., State University of Iowa, Iowa City, Iowa.

Kleinholz, Lewis H., Department of Biology, Reed College, Portland, Oregon.

Klotz, Dr. I. M., Department of Chemistry, Northwestern University, Evanston, Illinois.

Knowlton, Prof. F. P., 1356 Westmoreland Avenue, Syracuse, New York.

Kopac, Dr. M. J., Washington Square College. New York University, New York City, New York.

Krahl, Dr. M. E., Washington University School of Medicine, Department of Pharmacology, St. Louis, Missouri.

Krieg, Dr. Wendell J. S., 303 East Chicago Ave., Chicago, Illinois.

Lancefield, Dr. D. E., Queens College, Flushing, New York.

Lancefield, Dr. Rebecca C., Rockefeller Institute, New York City, New York.

Landis, Dr. E. M., Harvard Medical School, Boston, Massachusetts.

Lange, Dr. Mathilda M., Box 307, Central Valley, New York.

Lavin, Dr. George I., Rockefeller Institute, New York City, New York.

Lazarow, Dr. Arnold, Western Reserve University School of Medicine, Cleveland 6, Ohio.

Lee, Dr. Richard E., Cornell University College of Medicine, New York City, New York.

LeFevre, Paul G., Department of Physiology, University of Vermont College of Medicine, Burlington, Vermont.

Levy, Dr. Milton, Chemistry Department, New York University School of Medicine, New York City.

Lewis, Prof. I. F., University of Virginia, Charlottesville, Virginia.

Lillie, Prof. Ralph S., The University of Chicago, Chicago, Illinois.

Little, Dr. E. P., Harvard Computation Laboratory, Cambridge 38, Massachusetts.

Lochhead, Dr. John H., Department of Zoology, University of Vermont, Burlington, Vermont.

Loeb, Prof. Leo, 40 Crestwood Drive, St. Louis, Missouri.

Loeb, Dr. R. F., 180 Fort Washington Avenue, New York City, New York.

Loewi, Prof. Otto, 155 East 93d Street, New York City, New York.

Lowther, Mrs. Florence deL., Barnard College, Columbia University, New York City, New York.

Lucké, Prof. Balduin, University of Pennsylvania, Philadelphia, Pennsylvania.

Lynch, Dr. Clara J., Rockefeller Institute, New York City, New York.

Lynch, Dr. Ruth Stocking, Department of Botany, University of California, Los Angeles 24, California.

Lynn, Dr. William G., Department of Biology, The Catholic University of America, Washington, D. C.

MacDougall, Dr. Mary S., Agnes Scott College, Decatur, Georgia.

MacNaught, Mr. Frank M., Marine Biological Laboratory, Woods Hole, Massachusetts.

McCouch, Dr. Margaret Sumwalt, University of Pennsylvania Medical School, Philadelphia, Pa.

McDonald, Dr. Margaret H., Carnegie Institute of Washington, Cold Spring Harbor, Long Island, New York.

McGregor, Dr. J. H., Columbia University, New York City, New York.

Macklin, Dr. Charles C., School of Medicine, University of Western Ontario, London, Canada.

Magruder, Dr. Samuel R., Department of Anatomy, Tufts Medical School, Boston, Massachusetts.

Malone, Prof. E. F., 153 Cortland Avenue, Winter Park, Florida.

Marmont, Dr. George H., Institute of Radiobiology and Biophysics, University of Chicago, Chicago, Illinois.

Marshak, Dr. A., Department of Biochemistry, New York University College of Medicine, New York City, New York.

Manwell, Dr. Reginald D., Syracuse University, Syracuse, New York.

Marsland, Dr. Douglas A., Washington Square College, New York University, New York City, New York.

Martin, Prof. E. A., Department of Biology, Brooklyn College, Brooklyn, New York.

Mathews, Prof. A. P., Woods Hole, Massachusetts.

Matthews, Dr. Samuel A., Thompson Biological Laboratory, Williams College, Williamstown, Massachusetts.

Mavor, Prof. James W., 8 Gracewood Park, Cambridge 58, Massachusetts.

Mazia, Dr. Daniel, University of Missouri, Department of Zoology, Columbia, Missouri.

Medes, Dr. Grace, Lankenau Research Institute, Philadelphia, Pennsylvania.

Meigs, Mrs. E. B., 1736 M Street, N.W., Washington, D. C.

Memhard, Mr. A. R., Riverside, Connecticut.

Menkin, Dr. Valy, Department of Surgical Research, Temple University Medical School, Philadelphia, Pennsylvania.

Metz, Dr. C. B., Osborn Zoological Laboratory, Yale University, New Haven, Connecticut.

Metz, Prof. Charles W., University of Pennsylvania, Philadelphia, Pennsylvania.

Miller, Dr. J. A., 106 Forrest Avenue, N.E., Atlanta 3, Georgia.

Milne, Dr. Lorus J., Zoology Department, University of New Hampshire, Durham, New Hampshire.

Minnich, Prof. D. E., Department of Zoology, University of Minnesota, Minneapolis, Minnesota.

Mitchell, Dr. Philip H., Woods Hole, Mass.

Moore, Dr. Carl R., The University of Chicago, Chicago, Illinois.

Moore, Dr. J. A., Barnard College, New York City, New York.

Moul, Dr. E. T., Department of Botany, Rutgers University, New Brunswick, New Jersey.

Mountain, Mrs. J. D., 9 Coolidge Avenue, White Plains, New York.

Muller, Prof. H. J., Department of Zoology, Indiana University, Bloomington, Indiana.

Nabrit, Dr. S. M., Atlanta University, Morehouse College, Atlanta, Georgia.

Nachmansohn, Dr. D., College of Physicians and Surgeons, New York City, New York.

Navez, Dr. Albert E., 206 Churchill's Lane, Milton 86, Massachusetts.

Neurath, Dr. H., Department of Biochemistry, Duke University School of Medicine, Durham, North Carolina.

Newman, Prof. H. H., 173 Devon Drive, Clearwater, Florida.

Nichols, Dr. M. Louise, Rosemont, Pennsylvania.

Northrop, Dr. John H., Department of Bacteriology, University of California, Berkeley 4, California.

Ochoa, Dr. Severo, New York University, College of Medicine, New York 16, New York.

Oppenheimer, Dr. Jane M., Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania.

Osborn, Prof. R. C., Ohio State University, Columbus, Ohio.

Oster, Dr. Robert H., University of Maryland, School of Medicine, Baltimore, Maryland.

Osterhout, Prof. W. J. V., Rockefeller Institute, New York City, New York.

Osterhout, Mrs. Marian Irwin, Rockefeller Institute, New York City, New York.

Packard, Dr. Charles, Marine Biological Laboratory, Woods Hole, Massachusetts.

Page, Dr. Irvine H., Cleveland Clinic, Cleveland, Ohio.

Pappenheimer, Dr. A. M., 45 Holden Street, Cambridge, Massachusetts.

Parker, Prof. G. H., Harvard University, Cambridge, Massachusetts.

Parmenter, Dr. C. L., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.

Parpart, Dr. Arthur K., Princeton University, Princeton, New Jersey.

Patten, Dr. Bradley M., University of Michigan Medical School, Ann Arbor, Michigan.

Payne, Prof. F., University of Indiana, Bloomington, Indiana.

Peebles, Prof. Florence, 380 Rosemont Avenue, Pasadena, California.

Pierce, Dr. Madelene E., Vassar College, Poughkeepsie, New York.

Pinney, Dr. Mary E., Wilson, Kansas.

Plough, Prof. Harold H., Amherst College, Amherst, Massachusetts.

Pollister, Dr. A. W., Columbia University, New York City, New York.

Pond, Dr. Samuel E., 53 Alexander Street, Manchester, Connecticut.

Pratt, Dr. Frederick H., 105 Hundreds Road, Wellesley Hills 82, Massachusetts.

Prosser, Dr. C. Ladd, 345 Natural History Building, University of Illinois, Urbana, Illinois.

Ramsey, Dr. Robert W., University of Virginia Medical School, Richmond, Virginia.

Rand, Dr. Herbert W., 7 Siders Pond Road, Falmouth, Massachusetts.

Rankin, Dr. John S., Zoology Department, University of Connecticut, Storrs, Connecticut.

Redfield, Dr. Alfred C., Harvard University, Cambridge, Massachusetts.

Reid, Dr. W. M., Monmouth College, Monmouth, Illinois.

Reiner, Dr. J. M., Department of Physiology, Tufts College Medical School, Boston, Massachusetts.

Renn, Dr. Charles E., Sanitary Laboratories, The Johns Hopkins University Baltimore, Maryland.

Reznikoff, Dr. Paul, Cornell University Medical College, New York City, New York.

Rice, Prof. Edward L., Ohio Wesleyan University, Delaware, Ohio.

Richards, Prof. A., University of Oklahoma, Norman, Oklahoma.

Richards, Dr. A. Glenn, Entomology Department, University Farm, Univ. of Minnesota, St. Paul 8, Minnesota.

Richards, Dr. O. W., Research Department, American Optical Co., Buffalo, New York.

Riggs, Lawrason, 120 Broadway, New York City, New York.

Rogers, Prof. Charles G., Oberlin College, Oberlin, Ohio.

Rogick, Dr. Mary D., College of New Rochelle, New Rochelle, New York.

Romer, Dr. Alfred S., Harvard University, Cambridge, Massachusetts.

Root, Dr. R. W., Department of Biology, College of the City of New York, New York City, New York.

Root, Dr. W. S., College of Physicians and Surgeons, Department of Physiology, New York City, New York.

Rose, Dr. S. Meryl, Department of Zoology, University of Illinois, Urbana, Illinois.

Rothenberg, Dr. M. A., Institute of Radiobiology and Biophysics, University of Chicago, Chicago, Illinois.

Ruebush, Dr. T. K., 9712 Elrod Road, Kensington, Maryland.

Rugh, Dr. Roberts, Radiological Research Laboratory, College of Physicians and Surgeons, New York, N. Y.

Ryan, Dr. Francis J., Columbia University, New York City, N. Y.

Sampson, Dr. Myra M., Smith College, Northampton, Massachusetts.

Saslow, Dr. George, Washington University Medical School, St. Louis, Missouri

Saunders, Lawrence, R. D. 7, Bryn Mawr, Pennsylvania.

Schaeffer, Dr. Asa A., Biology Department, Temple University, Philadelphia, Pennsylvania.

Scharrer, Dr. Ernst A., Department of Anatomy, University of Colorado School of Medicine and Hospitals, Denver, Colorado.

Schechter, Dr. Victor, College of the City of New York, New York City, New York.

Schmidt, Dr. L. H., Christ Hospital, Cincinnati, Ohio.

Schmitt, Prof. F. O., Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Schmitt, Dr. O. H., Department of Physics, University of Minnesota, Minneapolis 14, Minnesota.

Scholander, Dr. P. F., Harvard Medical School, Department of Biochemistry, Boston, Massachusetts.

Schotté, Dr. Oscar E., Department of Biology, Amherst College, Amherst, Massachusetts.

Schrader, Dr. Franz, Department of Zoology, Columbia University, New York City, New York.

Schrader, Dr. Sally Hughes, Department of Zoology, Columbia University, New York City, New York.

Schramm, Prof. J. R., University of Pennsylvania, Philadelphia, Pennsylvania.

Scott, Dr. Allan C., Union College, Schenectady, New York.

Scott, Sister Florence Marie, Professor of Biology, Seton Hill College, Greensburg, Pennsylvania.

Scott, Dr. George T., Oberlin College, Oberlin, Ohio.

Semple, Dr. R. Bowling, 140 Columbia Heights, Brooklyn, New York.

Severinghaus, Dr. Aura E., Department of Anatomy, College of Physicians and Surgeons, New York City, New York.

Shanes, Dr. Abraham M., Department of Physiology and Biophysics, Georgetown University, School of Medicine, Washington, D. C.

Shapiro, Dr. Herbert, The Henry Phipps Institute, 7th and Lombard Sts., Philadelphia 47, Pennsylvania.

Shull, Prof. A. Franklin, University of Michigan, Ann Arbor, Michigan.

Shumway, Dr. Waldo, Stevens Institute of Technology, Hoboken, New Jersey.

Sichel, Dr. Ferdinand J. M., University of Vermont, Burlington, Vermont.

Sichel, Mrs. F. J. M., 35 Henderson Terrace, Burlington, Vermont.

Sinnott, Dr. E. W., Osborn Botanical Laboratory, Yale University, New Haven, Connecticut.

Slifer, Dr. Eleanor H., Department of Zoology, State University of Iowa, Iowa City, Iowa.

Smith, Dr. Dietrich Conrad, Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland.

Snyder, Prof. L. H., University of Oklahoma, Norman, Oklahoma.

Sonneborn, Dr. T. M., Department of Zoology, Indiana University, Bloomington, Indiana.

Speidel, Dr. Carl C., University of Virginia, University, Virginia.

Steinbach, Dr. Henry Burr, University of Minnesota, Minneapolis, Minnesota.

Stern, Dr. Kurt G., Polytechnic Institute, Department of Chemistry, Brooklyn New York.

Stewart, Dr. Dorothy, Rockford College, Rockford, Illinois.

Stokey, Dr. Alma G., Department of Botany, Mount Holyoke College, South Hadley, Massachusetts.

Straus, Dr. W. L., Johns Hopkins Medical School, Baltimore, Maryland.

Stunkard, Dr. Horace W., New York University, University Heights, New York City, New York.

Sturtevant, Dr. Alfred H., California Institute of Technology, Pasadena, California.

Szent-Györgyi, Dr. A. E., National Health Institute, Department of Biophysics, Bethesda, Maryland.

TASHIRO, DR. SHIRO, Medical College, University of Cincinnati, Cincinnati, Ohio.
TAYLOR, DR. WILLIAM R., University of Michigan, Ann Arbor, Michigan.
TEWINKEL, DR. L. E., Department of Zoology, Smith College, Northampton, Massachusetts.
TRACY, DR. HENRY C., University of Kansas, Lawrence, Kansas.
TRAGER, DR. WILLIAM, Rockefeller Institute, Princeton, New Jersey.
TRINKAUS, DR. J. PHILIP, Department of Zoology, Osborn Zoological Laboratory, New Haven, Connecticut.
TURNER, DR. ABBY H., Mt. Holyoke College, South Hadley, Massachusetts.
TURNER, PROF. C. L., Northwestern University, Evanston, Illinois.
TYLER, DR. ALBERT, California Institute of Technology, Pasadena, California.
UHLENHUTH, DR. EDUARD, University of Maryland, School of Medicine, Baltimore, Maryland.
VILLEE, DR. CLAUDE A., JR., Harvard Medical School, Boston, Massachusetts.
VISSCHER, DR. J. PAUL, Western Reserve University, Cleveland, Ohio.
WAINIO, DR. W. W., Bureau Biological Research, Rutgers University, New Brunswick, New Jersey.
WALD, DR. GEORGE, Biological Laboratories, Harvard University, Cambridge, Massachusetts.
WARBASSE, DR. JAMES P., Woods Hole, Massachusetts.
WARNER, DR. ROBERT C., Department of Chemistry, New York University College of Medicine, New York 16, New York.
WARREN, DR. HERBERT S., 1405 Greywall Lane, Overbrook Hills, Philadelphia 31, Pennsylvania.
WATERMAN, DR. ALLYN J., Department of Biology, Williams College, Williamstown, Massachusetts.
WATERMAN, DR. T. H., Osborn Zoological Laboratory, Yale University, New Haven, Connecticut.
WEISS, DR. PAUL A., Department of Zoology, The University of Chicago, Chicago, Illinois.
WENRICH, DR. D. H., University of Pennsylvania, Philadelphia, Pennsylvania.
WHEDON, DR. A. D., North Dakota Agricultural College, Fargo, North Dakota.
WHITAKER, DR. DOUGLAS M., Stanford University, California.
WHITE, DR. E. GRACE, Wilson College, Chambersburg, Pennsylvania.
WHITING, DR. ANNA R., University of Pennsylvania, Philadelphia, Pennsylvania.
WHITING, DR. PHINEAS W., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.
WICHTERMAN, DR. RALPH, Biology Department, Temple University, Philadelphia, Pennsylvania.
WIEMAN, PROF. H. L., University of Cincinnati, Cincinnati, Ohio.
WILBER, DR. C. G., St. Louis University, Department of Biology, St. Louis 4, Missouri.
WILLIER, DR. B. H., Department of Biology, Johns Hopkins University, Baltimore, Maryland.
WILSON, DR. J. W., Brown University, Providence, Rhode Island.
WITSCHI, PROF. EMIL, Department of Zoology, State University of Iowa, Iowa City, Iowa.

Wolf, Dr. Ernst, Pendleton Hall, Wellesley College, Wellesley, Massachusetts.

Woodward, Dr. Alvalyn E., Zoology Department, University of Michigan, Ann Arbor, Michigan.

Wrinch, Dr. Dorothy, Smith College, Northampton, Massachusetts.

Yntema, Dr. C. L., Department of Anatomy, Syracuse University Medical College, Syracuse, New York.

Young, Dr. B. P., Cornell University, Ithaca, New York.

Young, Dr. D. B., 7128 Hampden Lane, Bethesda, Maryland.

Zinn, Dr. Donald J., Box 66, State College, Kingston, Rhode Island.

Zorzoli, Dr. Anita, Department of Physiology, Washington University School of Dentistry, St. Louis 10, Missouri.

Zwilling, Dr. E., Department of Genetics, University of Connecticut, Storrs, Connecticut.

3. Associates of the Marine Biological Laboratory

Adler, Mrs. Cyrus
Adler, Dr. and Mrs. F. H.
Allen, Mr. and Mrs. Eugene Y.
Bailey, Mr. and Mrs.
Bartow, Mrs. Francis D.
Behnke, Mr. John A.
Bell, Mrs. Arthur
Brown, Mrs. Theodore E.
Buffington, Mr. and Mrs. George
Burk, Dr. and Mrs. Dean
Cahoon, Mr. and Mrs. Sam
Calkins, Mrs. Gary N.
Claff, Mrs. C. Lloyd
Clark, Mr. Alfred Hull
Clowes, Mrs. G. H. A.
Cooper, Mr. and Mrs. Charles P.
Crane, Mrs. Frances A.
Crane, Mrs. Murray
Crane, Mr. Richard
Crossley, Mr. and Mrs. Archibald
Crowell, Mr. Prince S.
Curtis, Dr. and Mrs. W. D.
Daniels, Mr. and Mrs. Harold
Draper, Mrs. Mary C.
Drinker, Dr. and Mrs. Cecil
Elsmith, Mrs. Dorothy
Enders, Mr. Fred
Fay, Mr. Henry H.
Fisher, Mrs. Bruce Crane
Frost, Mrs. Frank
Gannett, Mr. and Mrs. Robert T.
Garfield, Mrs. I. McD.
Gifford, Mr. and Mrs. John A.
Grant, Mrs. Marjorie S. M.
Greene, Mr. George
Greene, Miss Gladys M.
Hall, Mr. and Mrs. Henry
Howe, Mrs. Harrison
Hunt, Mrs. Reid
Janney, Mrs. Walter C.
Jewett, Mr. George F.
Keep, Mr. and Mrs. Frederic A.
Keith, Mr. and Mrs. Harold C.
Kidder, Mr. and Mrs. Henry M.
Kidder, Mrs. Jerome
Knower, Mrs. Henry
Koller, Mrs. Lewis
Lillie, Mrs. Frank R.
Lillie, Mrs. Ralph S.
Marvin, Mrs. A. H.
Mather, Dr. and Mrs. Frank J.
Meigs, Mrs. Edward B.
Mitchell, Mrs. James McC.
Mixter, Mrs. Jason
Montgomery, Mrs. T. H.
Moore, Mrs. William A.
Morgan, Mrs. T. H.
Morrison, Mr. David
Motley, Mrs. Thomas
Murphy, Dr. Walter J.
Newton, Miss Helen
Nims, Mr. and Mrs. E. D.
Norman, Mr. and Mrs. Edward

Oppenheim-Errer, Dr. and Mrs. Paul
Oster, Mrs. R. H.
Packard, Dr. and Mrs. L. B.
Park, Mr. Malcolm
Ratcliffe, Mrs. Thomas G.
Redfield, Mrs. Alfred
Rentschler, Mr. and Mrs. George
Reznikoff, Mrs. Paul
Richards, Mrs. A. N.
Riggs, Mrs. Lawrason
Rudd, Mrs. H. W. Dwight
Sands, Mrs. Adelaide
Saunders, Mrs. Lawrence
Spackman, Miss Emily S.
Spivack, Mr. Leo H.
Springer, Mr. and Mrs. Otto
Steel, Mr. Richard
Stockard, Mrs. Charles R.
Strecher, Mrs.
Strong, Miss Jane
Strong, Dr. Oliver S.
Swift, Mrs. Katherine W.
Swope, Mr. Gerard
Szent-Gyorgyi, Mrs. Albert
Tebbetts, Mr. and Mrs. Walter
Ward, Mr. and Mrs. Francis T.
Webster, Mrs. Edwin S.
Wick, Mrs. Myron A.
Wickersham, Mr. and Mrs. James H.
Williston, Professor Samuel
Wilson, Mrs. Edmund B.
Wolfinsohn, Mrs. Wolfe

A CYTOLOGICAL AND CYTOCHEMICAL STUDY OF THE MALE ACCESSORY REPRODUCTIVE GLANDS IN THE JAPANESE BEETLE, POPILLIA JAPONICA NEWMAN

JOHN MAXWELL ANDERSON

Arnold Biological Laboratory, Brown University

Introduction

These studies were undertaken in order to investigate the cytological details of the accessory reproductive glands of Popillia, and to determine whether regional cytological differences could be correlated with differences in secretory activity. Some of the techniques of cytochemistry have been applied to these glands to elucidate the general nature of the secretion product, and to localize sites of origin of its components.

The male genital system of insects is typically characterized by the presence of a varying number of accessory glands appended to the principal effluent passages. These have been variously considered as analogous to Cowper's gland and the prostate of vertebrates (Hegetschweiler, 1820) and as seminal vesicles (Dufour, 1824). Escherich (1894) correctly described the functions and embryonic origins of the accessory glands of several Coleoptera. Those of Lucanus (a Scarabaeoid beetle related to Popillia) were shown to be of mesodermal origin. Escherich had demonstrated (1893) the similarities between the male genital tract of Lucanus and those of the Scarabaeidae. The conclusions of Escherich were substantiated by Blatter (1897) in a histological study of the accessory glands of Hydrophilus. Comparative studies of the male genital system in a large number of Coleoptera by Bordas (1900) elucidated the features of this system with its accessories and described the characteristic appearance of the accessory gland secretions. Studies by Rittershaus (1927) of the structure and biology of Anomala and Phyllopertha dealt briefly with the structure and function of the accessory glands of the male. The glands of these species, which are members of the same family (Rutelidae) as Popillia closely resemble those of Popillia. In these beetles the secretion product of the accessory glands serves as a vehicle for sperm transfer and coagulates in the female tract to form spermatophores.

Material and Methods

The entire reproductive tract was dissected from adult male Japanese beetles. The glands were carefully teased from their positions along the gut wall and allowed to fall into loose coils about the testicular masses. The systems thus removed were fixed and subsequently treated in a variety of ways, depending upon the particular structures or substances to be demonstrated. The specific techniques used will be summarized below in connection with the observations.

It is disadvantageous to handle the material as entire reproductive tracts because the coils of the gland become disposed at random about the remainder of the organs. Since in many of the techniques used it is advisable to employ adjacent slides as controls, it often happens that control and test slides contain sections of different regions of the gland. As a result, some levels have eluded treatment with some of the techniques. Indications will be given below of regions for which specific data are lacking.

OBSERVATIONS

A. General morphological considerations

GROSS ANATOMY AND RELATIONSHIPS. There are two accessory reproductive glands in the adult male. They are long, coiled tubes which lie closely applied to the wall of the gut. Their length is approximately 4 times that of the abdomen, although, since they are extremely delicate, it is difficult to extend them fully. In the living animal the glands are a yellowish color. The small apical portion (diameter about 65 μ) tapers gradually to an expanded region (diameter about 246 μ) at the basal portion of the gland. The expanded sac-like structures continue posteriorly to the point at which the two glands join to receive the vasa deferentia and form the ejaculatory duct.

MICROSCOPIC ANATOMY. The following observations were made on material fixed in Flemming's strong solution, sectioned serially at 5 μ, and stained in iron-alum hematoxylin. Other glands fixed in Zenker-acetic were sectioned serially and stained with eosin-Y and methylene blue.

At its apical end, designated as Level I, each gland consists of an almost solid cylinder of moderately tall columnar cells 24 μ high and about 9 μ broad (Figs. 1, 16). Arranged radially, the cells rest upon a very thin basement membrane, and they bound a small, irregular lumen. In the lumen, a scanty, thin secretion lies upon the free ends of the cells. Each cell contains a large, ovoid nucleus near its basal end.

A short distance posteriorly, the diameter of the gland increases gradually to about 98 μ. The cells at this level (referred to as Level II) are of the same shape and size as those in Level I, but the diameter of the lumen has increased to approximately 60 μ (Fig. 7). The secretion in the lumen appears to differ little from that in Level I, but it is more abundant and contains numerous scattered small globules present in small numbers at Level 1. The separation of the upper region of the gland into Levels I and II is largely a matter of convenience, based chiefly on differences in diameter.

About midway between the apex and the enlarged basal portion of the gland, the diameter increases to 158 μ and the lumen measures approximately 88 μ (Level III). The cells are much taller than those in the upper regions (Figs. 3, 22), measuring about 46 by 10 μ. Most of them appear to be carrying on intense secretory activity. That the secretory process is of the apocrine type is indicated by globules of secretion-packed cytoplasm which are budded or pinched off from the apices into the lumen. Levels II and III are united by a short intermediate zone in which Level II gradually blends into Level III (Fig. 8).

At this zone of transition two layers of striated muscle are found, for the first time, outside the basement membrane. The outer layer consists of narrow, separate, parallel bands of longitudinal fibers; the inner, of an uninterrupted sheet of circular muscle. The total thickness of the muscle coats is 3 to 5 μ.

The accumulation of secretion in the lower regions of the gland lumen proceeds rapidly and exerts a marked influence upon the histology of the portion nearer the basal end. The increase in total diameter of the gland as it approaches the ejaculatory duct is accompanied by an increase in the diameter of the lumen and a decrease in the height of the epithelial cells. In the largest region of the gland (Level IV) the total diameter approximates 250 μ; the diameter of the lumen is 225 μ; the muscle coats remain 3 to 5 μ thick. The epithelial cells are cuboidal or squamous and measure about 10 μ in height. There is a gradual transition from Level III to Level IV. There is practically no evidence of secretory activity in cells typical of Level IV.

B. Special methods and observations

CYTOPLASMIC BASOPHILIA. Serial sections of material fixed in Zenker-acetic were stained with eosin-methylene blue. Adjacent sections were treated with ribonuclease [1] before staining.

The cells of all the levels of the gland show intense cytoplasmic basophilia. In Levels I and II, the cells have a dense perinuclear basophilic area from which radiate undulant basophilic strands. These fiber-like elements lie parallel to the longitudinal axis of the cell and pass around clear cytoplasmic areas above and below the nucleus (Fig. 10). The free secretion in the lumen consists of a uniformly acidophilic, granular mass of ground substance. In Level I it contains a few small, clear, unstained globules or vacuoles which are more numerous in Level II. Occasionally some parts of the ground-mass may stain with methylene blue, giving rise to a purplish rather than a blue tint (Fig. 7, edges of secretion mass).

The cells in Level III also show a perinuclear concentration of basophilia from which basophilic strands radiate toward the apices of the cells (Figs. 3, 8). The secretion droplets which are being shed from the apices of these cells are intensely and solidly basophilic. However, as these cytoplasmic globules break down and become parts of the free secretion mass their contents stain less intensely. The free secretion from this level down consists of an acidophilic granular ground substance which contains scattered clear globules (these two apparently produced in Levels I and II), and numerous somewhat basophilic larger elements produced in Level III.

The cells in the largest region of the gland show none of the streaming basophilic fibrillar structures characteristic of other levels. In these cells (Level IV), the nucleus is capped proximally and distally by strongly basophilic masses (Figs. 3, 4, 5). These cells show no evidence of secretory activity.

After treatment with ribonuclease all traces of cytoplasmic basophilia are removed. The strands or fibers remain as pale, acidophilic "ghosts" in the cytoplasm, although no remnants of the perinuclear concentrations persist (Figs. 2, 6, 11). The basophilic elements free in the lumen, however, are not affected by the enzyme (Figs. 4, 6).

Nuclear basophilia is virtually unchanged by ribonuclease, with the exception of the two or more large, conspicuous nucleoli which become partially acidophilic;

---

[1] Supplied by Dr. William Montagna of this laboratory.

these give the impression of a basophilic shell which encloses an inner acidophilic mass (cf. nucleoli in Figs. 5, 6). In untreated control sections the nucleoli are deeply basophilic.

GLYCOGEN AND OTHER POLYSACCHARIDES. Material fixed in cold Rossman's fluid and sectioned serially at 5 μ was treated according to the technique of Hotchkiss (1948). Sections exposed to saliva to digest glycogen were processed simultaneously as controls, and for the demonstration of polysaccharides other than glycogen.

Aggregations of Schiff-positive granules appear in the cells of all levels of the gland. In Levels I and II these masses lie both below and above the nucleus and extend partway from the nuclear membrane toward the apex of the cell. These aggregations evidently occupy the spaces between the basophilic strands described above, and they are more numerous in Level I than in Level II. These elements give a rather weak color reaction when compared with that in the glycogen-rich cells of the neighboring fat-body. The granular secretion in the lumen also gives a weak, pink reaction with leucofuchsin, but the small clear droplets are negative.

At Level III the tall apocrine cells show aggregates of pink granules at their free ends, and only a few near the nucleus. The granules are particularly concentrated in the terminal protoplasmic processes, and these small masses appear to recolorize the Schiff reagent more thoroughly as they break down and become the larger bodies in the free secretion. These bodies, which correspond to the basophilic components noted in eosin-methylene blue preparations, stain a deep magenta with leucofuchsin. (Fig. 9).

PLATE I

All figures are of paraffin-embedded material sectioned at 5 μ. Magnifications given are of the figures as they appear here.

FIGURE 1. Basophilia, Level I, eosin-methylene blue. Note cytoplasmic strands streaming from juxtanuclear concentrations; nucleoli. 750 ×.

FIGURE 2. Basophilia, Level I, eosin-methylene blue after ribonuclease digestion. Note persistence of basophilia in rims of nucleoli; acidophilic "ghosts" of cytoplasmic strands. 750 ×.

FIGURE 3. Basophilia, Level III (upper), Level IV (lower); eosin-methylene blue. Note deep basophilia in protoplasmic processes of Level III cells; basophilic elements in free secretion. 750 ×.

FIGURE 4. Basophilia, Level IV, eosin-methylene blue. Note basophilic areas surrounding nuclear caps; basophilic secretion bodies. 330 ×.

FIGURE 5. Same cells shown in Figure 4. 750 ×.

FIGURE 6. Basophilia, Level IV, eosin-methylene blue after ribonuclease digestion. Note disappearance of nuclear caps; persistence of basophilic rims of nucleoli and of basophilic elements in free secretion mass. 330 ×.

FIGURE 7. Basophilia, Level II. 330 ×.

FIGURE 8. Basophilia, transition zone Level II–III. Note first appearance of basophilic bodies in secretion mass.

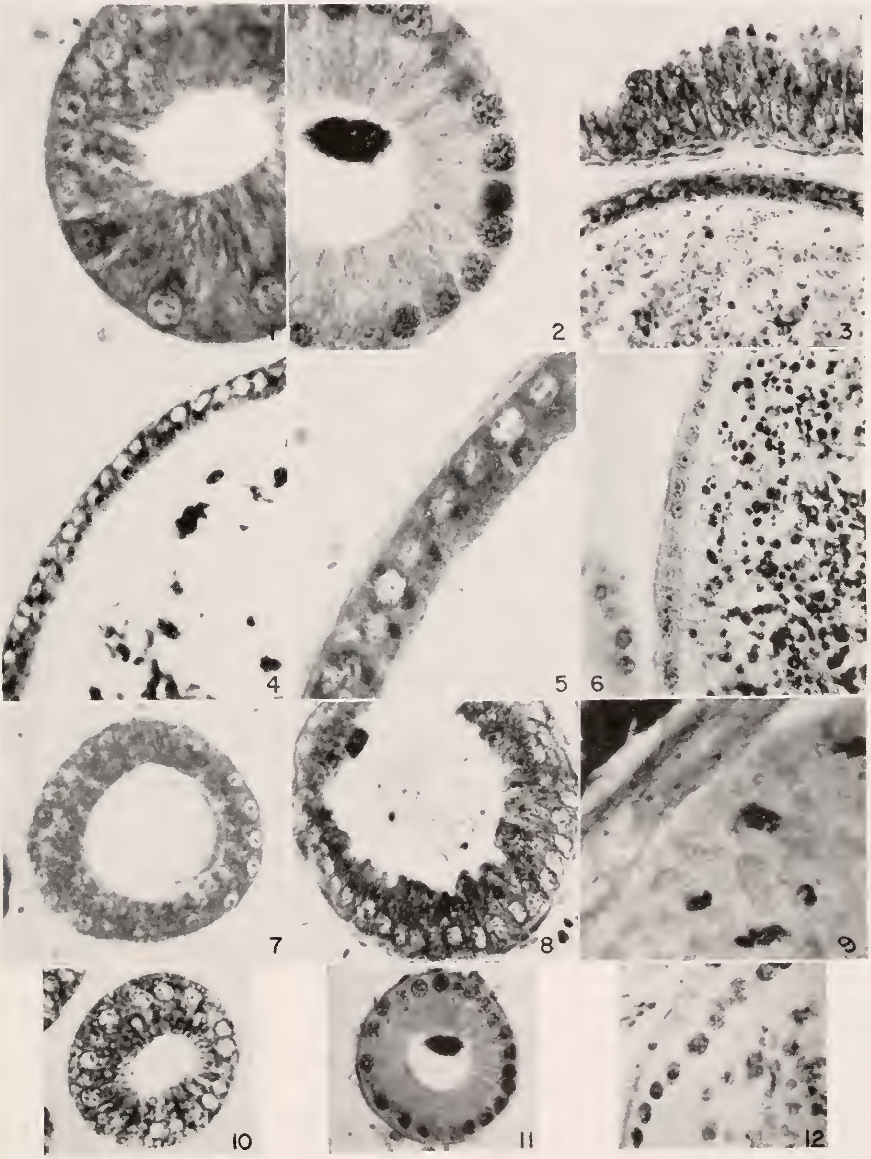
FIGURE 9. Periodic-acid-Schiff reaction, Level IV. Note glycogen bodies in cells; deeply-stained mucus flocs in secretion. Dark mass at upper left corner is glycogen in adjacent fat-body cells. 750 ×.

FIGURE 10. Basophilia, Level I–II; as in Figure 1. Note clear cytoplasmic areas. 330 ×.

FIGURE 11. Basophilia, Level I, after ribonuclease digestion. Same section as in Figure 2. 330 ×.

FIGURE 12. Toluidine blue reaction, Level IV. Note metachromatic flocs in secretion; ground-mass of secretion is greenish. 330 ×.

Plate I


1
2
3
4
5
6
7
8
9
10
11
12

At the largest level of the gland the cuboidal cells contain few granules comparable with those in upper levels. Each cell shows, near its nucleus, a few moderate-sized masses which stain intensely, and a few smaller granules near the free border which stain less deeply (Fig. 9). The free secretion at this level does not differ from that seen at Level III.

Except for the moderate-sized masses in cells at Level IV, none of the elements described above is destroyed by salivary digestion prior to treatment with the Schiff reagent. The appearance of the secretion within the lumen is not visibly affected by salivary amylase.

METACHROMATIC SUBSTANCES. For the demonstration of metachromasia, material was fixed in basic lead acetate-formaldehyde, sectioned serially at 5 μ, and stained in 0.1 per cent toluidine blue in 1 per cent alcohol; these sections were dehydrated and washed in 95 per cent and absolute alcohol, and mounted in balsam. Parallel series were stained in alcoholic toluidine blue at pH 3.8 and in a similar solution of the stain buffered to pH 5.0. Control sections were incubated for 6 hours with hyaluronidase as recommended by Wislocki, Bunting, and Dempsey (1947) and then stained with toluidine blue as above.

In sections stained at pH 5.0, the secretion in the lumen remains unstained, and the cytoplasm of the cells at all levels shows a general faint metachromasia. This non-localized and apparently non-specific metachromasia is probably of no significance. In unbuffered solutions of toluidine blue at pH 3.8, the cytoplasm of the gland cells is basophilic, while the large bodies scattered in the free secretion at Level III and below (which, it will be recalled, are highly Schiff-positive) are strongly metachromatic (Fig. 12). The ground substance of the secretion mass stains a bright greenish-blue. The granules in the cytoplasm of the gland cells are not metachromatic; if they do stain green, like the ground-mass in the lumen, this color is masked by the basophilia of the surrounding cytoplasm.

Treatment with hyaluronidase has no observable effect on the metachromatic staining of these substances with toluidine blue.

LIPIDS. Several methods were used to demonstrate lipoid sustances in the accessory glands.

1. SUDAN BLACK: thin frozen sections of material fixed in calcium-formaldehyde and embedded in gelatin were treated with sudan black according to Baker's (1944) directions. Such treatment reveals, at all levels, a variety of discrete cytoplasmic elements. At Level I, dot-like and irregularly-shaped bodies lie scattered in the cytoplasm and gathered at the basal ends of the nuclei. Many of these blackened structures are of different sizes and form irregular rims about clear vacuoles (Fig. 13). In the cells in Level II the vacuoles are larger and generally more numerous, lying between the nucleus and the free border of each cell. At Level III (Fig. 14) these bodies are similar to those in Level II in distribution and quantity, and it is apparent that no sudanophilic elements are concentrated in the protoplasmic processes, although some vacuolate bodies lie near the free ends of the cells. In the lower regions of the gland the appearance of the blackened structures is similar to that in upper levels, but they are fewer in number and lie chiefly near the nuclei. Reaction of the free secretion in the lumen to sudan black treatment in-

dicates that small amounts of lipoid material are present at all levels; the secretion mass shows scattered small droplets of sudanophilic substance lying dispersed in the ground-mass. In Level II, rows of small blackened droplets appear just outside the free borders of the cells, indicating that Level II is probably the chief site of release of the lipoid elements in the secretion. However, lipoid droplets are present in the lumen at Level I also. These small sudanophilic droplets apparently correspond to the small clear globules which remain unstained by eosin-methylene blue and by the Schiff reagent.

2. SUDAN BLACK FOLLOWING POSTCHROMATION: material fixed in calcium-formaldehyde was treated with dichromate-calcium before embedded in gelatin, sectioned on the freezing microtome, and colored with sudan black and carmalum (Cain, 1947a).

This manipulation reveals much additional lipoid material in the gland cells. The cells near the apex of the gland contain massive clumps of sudanophilic material which occupy almost the entire volume of the cytoplasm, above and below the nucleus (Fig. 16). Cells in Level II are similarly rich in sudanophilic elements, but these become less numerous and more scattered in the cytoplasm at the transition zone between Levels II and III. In this zone, and in Level III, the majority of the sudanophilic elements are clearly vacuolated, and they lie chiefly in clumps above and below the nucleus (Figs. 17, 18). Level IV cells show small clumps of blackened vacuolated bodies near their nuclei.

The free secretion is more sudanophilic after postchromation; many additional small to moderate-sized lipid droplets, some in Level I but more numerous in Level II and below, are revealed (Figs. 16–18).

3. ACID HEMATEIN ROUTINE: phospholipines were demonstrated by Baker's (1946) acid hematein test. This technique gives results essentially similar to those obtained by the use of Cain's postchromed-sudan black method, except that in the cells below Level II fewer cytoplasmic structures are colored. The crowded clumps previously noted in cells at Level I and upper Level II stain a deep blue to blue-black with acid hematein (Fig. 15). Unfortunately, only sections of the upper and lower transition zones of Level III have been found in these preparations; in the former, scattered vacuolated structures showing some concentration toward the nucleus are in evidence. Near the beginning of Level IV, the cells show positively-staining perinuclear elements which extend toward the free border (Fig. 21). Cells typical of Level IV present, very clearly, concentrated small globular aggregates at the upper ends of their nuclei, and some globules below the nuclei.

The free secretion shows some small, blue-black globules at all levels; these are more numerous below Level I. Some of the larger elements of the secretion stain sporadically a dark blue, the ground-mass, generally brown to yellow (negative), and the remaining large hyaline areas (Fig. 21) do not stain at all.

4. ACID HEMATEIN FOLLOWING PYRIDINE EXTRACTION: this is the control procedure for the acid hematein test for phospholipine (Baker, 1946). In the accessory reproductive glands, none of the cytoplasmic acid hematein-positive elements are present after the application of the pyridine extraction test (cf. Fig. 19 with 15, 22 with 21). This, according to Baker (1946) and Cain (1947 b), identifies the cytoplasmic acid hematein-positive substances as phospholipine. It may thus be

concluded that all cytoplasmic bodies stained by acid hematein in this material contain phospholipine.

Comparison of the reaction of the free secretion to acid hematein before and after pyridine extraction reveals that some, but not all, of the reactivity of the globules is removed by the action of the solvent (cf. secretion mass, Figs. 21 and 22). This substantiates the result of treatment with sudan black, which showed that some lipid was present in the secretion product; the acid hematein test further demonstrates that at least some of the lipid in the secretion consists of phospholipine. Since considerable amounts of residual secretion stain with acid hematein even after exposure to pyridine, particularly in the moderate to large bodies, this bears out the other evidence that some of it is non-lipoid.

5. OSMICATION TECHNIQUES: treatment of this material with such techniques as that of Worley (1946) reveals vacuolar and dot-like structures within the gland cells, more or less similar to those described after other methods. Some small to medium-sized secretion bodies in the lumen are intensely impregnated by this technique; in general, however, they do not correspond to the sudanophilic and acid hematein-positive bodies. The usual unreliable nature of osmication techniques makes it difficult to interpret the nature of these impregnated elements.

ACID-FAST SUBSTANCES. The distribution of the weakly Schiff-positive granules at both ends of the nuclei in the secretory cells suggested the possibility that they might consist of pigments. Therefore, 5 μ paraffin serial sections of material fixed in Zenker-acetic were subjected to staining with Verhoeff's carbol-fuchsin methylene blue for the demonstration of acid-fast material (Lillie, 1948). The granular structures gave a weak, indefinitely pink reaction to this technique also; this cannot be considered a positive reaction as compared with the behavior of certain intensely acid-fast granules in neighboring fat-body cells.

PLATE II

All figures are of gelatin-embedded material sectioned on the freezing microtome.

FIGURE 13. Sudanophilic structures, Level I; sudan black, carmalum. Note scattered globular and vacuolar bodies. 750 ×.

FIGURE 14. Sudanophilic structures, Level III; sudan black, carmalum. Note scattered vacuole-containing bodies between nucleus and free border. 750 ×.

FIGURE 15. Acid hematein, Level I. Note masses of formed bodies; scattered stained globules in secretion. 330 ×.

FIGURE 16. Sudanophilic structures, Level I; sudan black after postchromation. Cf. picture with Figures 13 and 15. 330 ×.

FIGURE 17. Sudanophilia after postchromation, upper end of transition zone, Level II–III. Cf. abundance and distribution of blackened bodies with that in Figure 16. Note sudanophilic elements in secretion mass. 330 ×.

FIGURE 18. Portion of same section as in Figure 17, 750 ×. Note concentration of Golgi vacuoles at nuclear caps, progression toward free border.

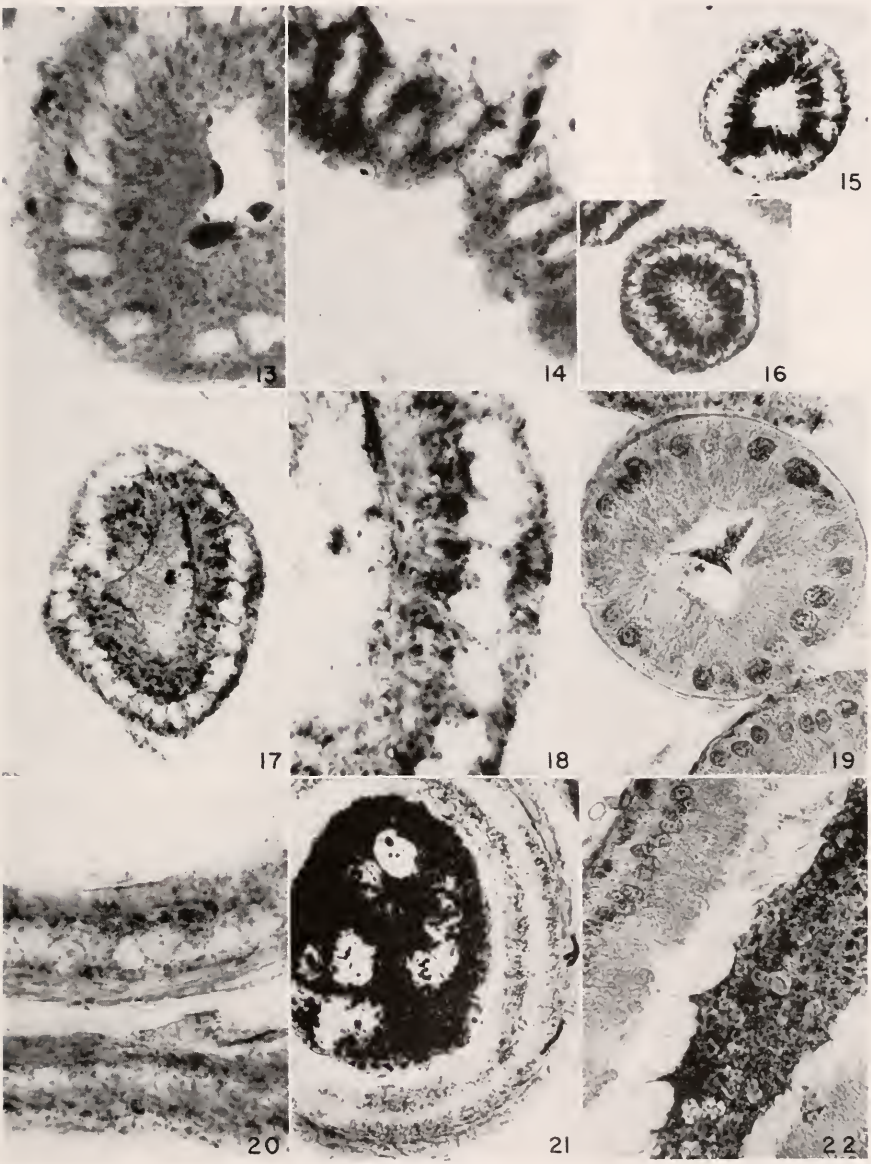
FIGURE 19. Acid hematein after pyridine extraction. Cf. Fig. 15. 750 ×.

FIGURE 20. Acid hematein, Level IV. Note aggregations of vacuole-containing Golgi-bodies at nuclear caps (cf. basophilic areas, Fig. 5). 750 ×.

FIGURE 21. Acid hematein, transition zone, Level III–IV. Note stained structures at nuclear caps, progression toward free border; deeply-stained bodies in free secretion; large clear areas in secretion mass. 330 ×.

FIGURE 22. Acid hematein after pyridine extraction, Level III. Cf. Figure 21 for removal of all cytoplasmic stained structures, loss of some staining capacity in free secretion. 330 ×.

PLATE II


13
14
15
16
17
18
19
20
21
22

It seems pertinent, at this time, to add a note concerning the appearance of the secretion product in the lumen after various fixatives. The variation in the appearance of the secretion mass following exposure to different fixatives makes it rather difficult to be certain of the correspondence of particular constituents, on the basis of size and appearance, in comparing results of various tests. In general, following treatment with strongly alcoholic fixatives (*e.g.*, Rossman, Zenker), the secretion product appears to consist of a finely-granular ground substance, somewhat shrunken away from the cells of its origin, containing scattered flocculent masses, some small clear vacuoles, and a few larger vacuolar structures. However, after exposure to such comparatively mild fixing fluids as calcium-formaldehyde, the ground substance appears as a mass of small, somewhat hyaline globular structures, bearing scattered larger globules or vacuoles of various sizes; under these conditions the mass shows much less tendency to shrink away from the walls of the gland. This behavior suggests that the ground material and the flocculent masses are composed of substances which tend to imbibe water and to swell, and that the milder fixatives permit this to occur to a certain extent before coagulation supervenes. The rapid action of the strongly alcoholic fixatives precipitates and coagulates the material before imbibition can occur. Alternatively, and perhaps more acceptably, it may be assumed that the normal condition of the secretion in the living animal is more or less similar to that seen after fixation in calcium-formaldehyde, and that this picture is altered by the dehydrating and precipitating action of the stronger fixatives and subsequent treatment of the material with alcoholic fluids.

Discussion

The observations of previous investigators on the histological features of the accessory glands indicate that some similarities exist between the glands of Popillia and those of other more or less closely related beetles. Bordas (1900) described the glands of Lucanus and of Dorcus (family Lucanidae) as composed of a secretory epithelium containing elongate, cylindrical cells, with their protoplasm granular near the base and compact and fibrillar near the free border. These cells release their secretion products by rupture, and globules of this product were frequently seen attached to the cell by a pedicel. Features such as these are characteristic also of the gland of Popillia, but only in the relatively restricted area designated in the present study as Level III.

Rittershaus (1927), working with two members of the same family as that containing Popillia (Anomala and Phyllopertha, fam. Rutelidae), briefly described differences in the type of epithelium present in different regions of their glands. She differentiated between tall, columnar epithelial cells and cuboidal to flattened cells. The lumen of the gland was described by this author as small in the upper region, filled with protoplasmic processes of the epithelial cells; in the lower region, the lumen was said to be clearly expanded and filled with glandular secretion. Rittershaus assumed that the protoplasmic processes were signs of strong secretory activity on the part of the cylindrical cells.

The observations outlined above indicate that regional histological differences in the gland, accompanied by differences in secretory activity, are characteristic also of Popillia. A detailed study of the glands in this species reveals, however, that there are more than the two regions described by Rittershaus. The morphological differences noted in this study include variations in the diameter of the gland and of its lumen, differences in shape, size, and type of cells, and differences in the amount and nature of the secretion product occupying the lumen. It is apparent that the secretory processes in Levels I and II differ somewhat in characteristics and in type of product from those in Levels III, and that Level IV, as indicated by Rittershaus in other species, serves mainly as an expanded reservoir for the collection and retention of secretions elaborated in the upper regions of the gland.

Cytoplasmic basophilia of the type exhibited in cells of the gland at all levels has been established by numerous investigators, in other materials, as indicating sites of ribonucleoproteins (Brachet, 1940, 1947; Dempsey and Wislocki, 1945, 1946). Intense cytoplasmic basophilia removable by ribonuclease has been reported as characteristic of cells secreting large amounts of protein (Brachet, 1940, and Caspersson *et al.*, 1941, for acinar cells of pancreas; Noback and Montagna, 1947, for salivary gland cells and acinar pancreas cells). In addition, Greenstein (1944) states that cells which synthesize large quantities of protein characteristically have large nucleoli. Noback and Montagna (1947) demonstrated that one or more large nucleoli are present in the nuclei of acinar cells of the pancreas and salivary glands, and that these nucleoli contain ribonucleoprotein. Among others, Brachet (1940) and Dempsey and Wislocki (1945) have also localized ribonucleic acid in cell nucleoli. The present studies have demonstrated the presence of ribonucleoprotein in the cytoplasm and in the large nucleoli of cells of the accessory reproductive glands; this may be considered as an indication that the secretion product of these glands is at least partially protein in nature.

The differences in the shape and distribution of cytoplasmic ribonucleoprotein structures in cells at different levels in the gland are presumably correlated with differences in the secretory activities of the different regions. For example, in the upper levels, where all techniques reveal active secretion, basophilic elements stream from the nuclear membrane to the free border of the cell. In Level IV, however, where cells are evidently not active in secretion, the cytoplasmic nucleoproteins are restricted to a limited and concentrated area surrounding the nuclear caps. These same areas in cells at Level IV also contain lipoid bodies which resemble the Golgi element (vide infra).

Failure to demonstrate appreciable amounts of stored glycogen in cells at any level except the lowest portion of the gland may also be taken as an indication that Level IV is not a secretory region. Other polysaccharides, Schiff-positive after periodic acid but not removed by salivary digestion, are present in accumulations between the nuclei and the free borders of actively secreting cells in the upper levels, but these are present in much smaller amounts in the cells of Level IV. These accumulations lie in cytoplasmic regions which show no affinity for methylene blue; they may be regarded, in view of the apparent polysaccharide nature of the released secretion (see below), as precursors of the secretory products of the cells in which they lie. No regional differences in their cytologic localization are apparent, except their paucity at Level IV.

It is difficult to determine the exact nature of the cytoplasmic structures colored by sudan black without post chromation. However, previous work in germ cells of Popillia has demonstrated that both mitochondria and Golgi-derivatives are revealed by such treatment (unpublished results). The scattered dot-like and vacuole-containing structures shown in cells of the accessory gland by this technique may represent mitochondria and some dispersed and poorly-preserved constituents of the Golgi element. In tissues so treated the cells at any level do not show concentrated juxtanuclear groups of Golgi vacuoles. In thin frozen sections there is a progression of vacuolated sudanophilic structures from the nuclear membrane to the free border, and an apparent increase in size of the vacuoles as they approach the apex of the cell. These things may indicate that these elements are like Golgi-bodies, and they presumably participate actively in secretory processes. Except for their scattered disposition and fewer numbers, they resemble the vacuolated elements demonstrated by sudan black after postchromation; they are like the constituents of the Golgi element described by Cain (1947a) in secretory cells of the alimentary canal of the leech Glossiphonia.

The striking regional differences in the abundance and distribution of lipid elements demonstrated by sudan black after postchromation, and by the acid hematein technique, are conspicuously at variance with the results of simple treatment with sudan black. With these techniques the dense masses of clearly localized droplets and globules shown in Levels I and II have no counterpart in cells at Level III and below. The massive and densely-packed nature of these elements makes it impossible to ascertain the exact morphological nature of the individual bodies. It seems probable, however, that they represent the more or less complete cellular complement of mitochondria and Golgi-bodies, some of which are lost in preparation unless the material is treated with potassium dichromate. The disparity in concentration of these elements between cells in Levels I and II and those in the lower regions of the gland suggests that secretory activity is greater in the upper levels. The appearance of the cytoplasmic phospholipines in these densely-packed cells does not indicate the occurrence of lipophanerosis, as described by Cain (1948) in certain neurones of Helix. This phenomenon was ascribed to a breakdown and release in the cytoplasm of all the cellular phospholipines, resulting in a tendency of the cytoplasm to stain uniformly. In the present instance the cellular phospholipines are not generally distributed, but they are definitely and discretely localized.

The appearance of the free secretion mass indicates that its lipoid components are produced in Levels I and II; these elements first appear in the free secretion in the areas of the gland characterized by an abundance of cytoplasmic phospholipines. It has been noted, (a) that globules of sudanophilic material are found in the secretion in Levels I and II; (b) that sudanophilic droplets lie in a fringe along the borders of cells in Level II, suggesting their having been released at this level; and (c) that certain of the components of the free secretion mass contain phospholipine. The possibility then remains that some of the cellular phospholipine demonstrated in the upper regions of the gland represents a phospholipine-containing product of secretory activity in these cells.

In areas of the gland where cytoplasmic lipoid structures are not obscured by overcrowding, it is clear that the nuclear caps which stain intensely with acid hematein represent the Golgi element as demonstrated by sudan black in post-chromed preparations. Using the acid hematein technique, several authors have previously shown that the Golgi-bodies of a variety of tissues contain phospholipine

(Cain, 1947a, for alimentary epithelium of Glossiphonia, and 1948, for nerve cells of Helix; Thomas, 1947, also for nerve cells in Helix; Montagna, Noback, and Zak, 1948, for sebaceous gland cells of man). The present studies show that the Golgi element in cells of the accessory reproductive gland of Popillia also contain phospholipine, along with other lipoid components which cause the vacuolated bodies to color more deeply and more generally with sudan black than with acid hematein.

The presence of the Golgi element within localized cytoplasmic areas which are surrounded by intensely basophilic zones, together with Schiff-positive granules and possibly phospholipine secretion-precursors, suggests that the Golgi-bodies are involved in the elaboration or accumulation of these products.

With regard to the general question of the nature of the secretion product of these glands, it is apparent that it is a mixture of a number of substances, present in the secretion mass as discrete components. Certain of these elements are produced in, and apparently traceable to, particular regions of the long, tubular glands. Within the limits of accuracy of correlating the varying appearances of secretion bodies after various methods of fixation, the following synopsis of their characteristics seems warranted, on the basis of their reactions to the different techniques employed.

1. THE GROUND SUBSTANCE: usually acidophilic; pink reaction with leucofuchsin, not removed by salivary digestion; greenish stain with toluidine blue at pH 3.8, not removed by alcohol; negative reaction with acid hematein test; not sudanophilic. Produced in Levels I and II.

2. SCATTERED SMALL TO MEDIUM-SIZED GLOBULES: neither acidophilic nor basophilic; negative reaction to leucofuchsin; no stain with toluidine blue; sudanophilic; positive reaction (at least in part) with acid hematein, negative after pyridine extraction; osmiophilic? Evidently produced in Levels I and II.

3. SCATTERED LARGER FLOCS OR GLOBULES: basophilic, not affected by ribonuclease; intense purplish stain with leucofuchsin, not removed by salivary digestion; purplish metachromasia with toluidine blue at pH 3.8, unaffected by alcohol or hyaluronidase; sometimes stains with acid hematein, but if so, not removed by pyridine extraction. Produced by apocrine cells in Level III.

On the basis of the characteristics listed, the scattered masses of highly Schiff-positive and metachromatic material described in 3, above, may be interpreted as consisting of mucus. McManus (1946, 1948) has demonstrated that mucus stored in and released from goblet cells in the intestine gives an intense color with leucofuchsin after periodic acid treatment. Wislocki, Bunting, and Dempsey (1947) found that mucus from several sources, including submaxillary glands of the guinea pig, and synovial fluid of rhesus monkeys, gave a strong reaction with the Bauer test. The Wislocki group (1947) found also that mucus gave a characteristically strong purplish metachromasia with toluidine blue, sometimes, but not always (depending on its source) abolished by hyaluronidase. Hempelmann (1940) demonstrated that mucoproteins from various sources, differing chemically, could be distinguished from one another on the basis of metachromatic reactions with toluidine blue and polychrome methylene blue. The secretion of the apocrine cells of the reproductive gland, being metachromatic and Schiff-positive, has the general characteristics of the group of mucoproteins indefinitely termed "mucus."

Other reactions of this material are also significant; in tests of the acid hematein routine using pure substances, Baker (1946) found that mucus occasionally stained, but that the staining reaction was not removed by treatment with pyridine. This is also true of the secretion-masses in the present case, which sometimes give a positive reaction with acid hematein but retain their reactivity after pyridine extraction. In addition, mucus, owing to its acid content, should be expected to show basophilia not removable by ribonuclease; the bodies under discussion show this characteristic.

The granular nature of the intracellular precursors of this secretion product in Popillia, and the evident tendency of the substance to swell upon being released from the cell, are also generally characteristic of mucus. The failure of the precursor-granules to give reactions like those of the released substance is interesting. Baker (1946) in his account of the reactions of various compounds with acid hematein found that, as described above, mucus outside of cells quite often gave a positive reaction, while mucin in the goblet cells of the mouse intestine consistently reacted negatively. Conditions within cells are sufficiently different from those outside cells to make it unreasonable to expect substances to react identically in both situations. However, it would appear that the mucus in the accessory gland secretion differs from the mucus of human jejunal goblet cells, for example, shown by McManus (1948) to be strongly Schiff-positive both before and after its release.

The production of this component of the secretion mass has been ascribed to cells in Level III because it never appears in the lumen above Level III but is characteristically abundant at this level and below.

Since the ground sustance (1, above) which makes up the bulk of the secretion recolors leucofuchsin, it must be related to mucus. That it is not identical with mucus is evident from the fact that its reaction with leucofuchsin is not so intense, and that it never gives a typical purple metachromasia with toluidine blue, but stains green. In addition, it seldom, if ever, reacts with acid hematein, and it is acidophilic. A tendency to stain green with toluidine blue has been reported by Wislocki, Bunting, and Dempsey (1947) for striated muscle fibers, thyroid colloid, stratum lucidum and stratum corneum of epidermis, and occasionally for nuclei. The substance so stained in the present instance would seem to be related in no obvious way to any of the examples cited. One may suggest, on the basis of the appearance of this substance and its reaction with leucofuchsin, that it is related to the mucus produced in Level III. It is probable that both substances are mucoproteins, members of a miscellaneous and widely-distributed group of compounds characterized as protein-polysaccharide complexes differing from one another chiefly in the nature of the polysaccharide moiety and its substituents (Hempelmann, 1940; Meyer, 1945). The periodic-acid-Schiff reaction, as pointed out by McManus (1948), cannot be used to distinguish actual chemical differences within this group of compounds.

The protein nature of secretions in both categories (1 and 3) is inferred from the abundance and intracellular distribution of ribonucleoproteins in cells at Levels I, II, and III, and by the conspicuousness of the ribonucleoprotein-containing nucleoli in cells at these levels.

The third recognizable constituent (2, above) is characterized as either entirely phospholipine or a mixture of phospholipine and other lipoidal substances. The site of its production has been indicated as Levels I and II; the characteristic droplets or globules of this product first appear in the lumen in these regions of the gland.

On the basis of the results obtained in this study, we may picture the secretion of the accessory reproductive glands as a viscous fluid, composed chiefly of different-sized globules of mucus and mucus-like polysaccharide-protein compounds, containing a certain amount of phospholipine and possibly other lipoids present as scattered droplets. Bordas (1900) described the secretion of these glands in the beetles Lucanus and Dorcus as homogeneous, compact, and "muqueuse," always gelatinous but coagulated by reagents into a hyaline mass. The secretion in Lucanus was described as showing (near the free borders of the cells) the presence of small, refringent globules, some adhering to cells and some free in the lumen. Rittershaus (1927) described and figured the appearance of the secretion mass in Anomala and Phyllopertha as consisting of a finely-granular ground substance containing larger clear bodies; the secretion was described as clumping together in the middle of the lumen and receiving additions of fresh secretion at its perifery.

A secretion product consisting of these components may be considered as admirably adapted to its several functions of lubrication, sperm-suspension, and spermatophore formation.

Summary

1. Differences in secretory activity have been correlated with morphological and cytological regional differences at successive levels in the accessory reproductive glands.

2. Intense cytoplasmic basophilia, abolished by ribonuclease, is characteristic of cells at all levels.

3. The Golgi element of the secretory cells is in the form of vacuolated spherules and is demonstrable by use of sudan black, particularly after postchromation. The Golgi element contains phospholipine.

4. The secretion product of the gland consists of a ground-mass of a mucus-like protein-polysaccharide compound, bearing scattered large globules of mucus and many smaller droplets of phospholipine, and possibly other lipoidal substances.

5. The mucus-like ground substance and the lipoid components are produced in the upper levels of the gland; the mucus globules originate in tall apocrine cells in the middle region; the lower levels apparently do not contribute to the secretion mass but serve as expanded reservoirs for the retention of the finished secretion product.

LITERATURE CITED

Baker, J. R., 1944. The structure and chemical composition of the Golgi element. *Quart. J. Micr. Sci.*, **85**: 1–71.

Baker, J. R., 1946. The histochemical recognition of lipine. *Quart. J. Micr. Sci.*, **87**: 441–470.

Blatter, P. 1897. Étude sur la structure histologique des glandes annexes de l'appareil mâle de l'Hydrophile. *Arch. d'Anat. Micr.*, **1**: 384–416.

Bordas, L., 1900. Recherches sur les organes reproducteurs males des Coléoptères (anatomie comparée, histologie, matière fécondante). *Ann. des Sciences Naturelles*, ser. 8, **11**: 283–448.

Brachet, J., 1940. La detection histochimique des acides pentosenucleiques. *C. R. Soc. Biol.*, **133**: 88–90.

Bracket, J., 1947. Embryologie Chimique. Masson & Cie., Paris.

Cain, A. J., 1947a. Demonstration of lipine in the Golgi apparatus in gut cells of Glossiphonia. *Quart. J. Micr. Sci.,* **88**: 151–157.

Cain, A. J., 1947b. An examination of Baker's acid hematein test for phospholipines. *Quart. J. Micr. Sci.,* **88**: 467–478.

Cain, A. J., 1948. The accumulation of carotenoids in the Golgi apparatus of neurones of Helix, Planorbis, and Limnaea. *Quart. J. Micr. Sci.,* **89**: 421–428.

Caspersson, T., H. Landström-Hyden and L. Aquilonius, 1941. Cytoplasma-nukleotide in Eiweissproduzierenden Drüsenzellen. *Chromosoma,* **2**: 111–131 (not available in original; quoted from Noback and Montagna, 1947).

Dempsey, E. W. and G. B. Wislocki, 1945. Histochemical reactions associated with basophilia and acidophilia in the placenta and pituitary gland. *Am. J. Anat.,* **76**: 277–301.

Dempsey, E. W. and G. B. Wislocki, 1946. Histochemical contributions to physiology. *Physiol. Rev.,* **26**: 1–27.

Dufour, L., 1824. Recherches anatomiques sur les Carabiques et sur plusieurs autres Insectes coléoptères. *Ann. des Sciences Naturelles,* **6**: 150–206.

Escherich, K., 1893. Ueber die verwandtschaftlichen Beziehungen zwischen den Lucaniden und Scarabaeiden. *Wien. Entomol. Zeitung,* **12**: 265–269.

Escherich, K., 1894. Anatomische Studien über das männliche Genital-system der Coleopteren. *Zeit. wissen. Zool.,* **57**: 620–641.

Greenstein, J. P., 1944. Nucleoproteins. Advances in Protein Chemistry. **1**: 210–288. Academic Press, Inc., N. Y.

Hegetschweiler, F., 1820. De Insectorum genitalibus. Inaug. Diss., Turici. (not seen in original; quoted from Escherich).

Hempelmann, L. H., Jr., 1940. Staining reactions of the mucoproteins. *Anat. Rec.,* **78**: 197–206.

Hotchkiss, R. D., 1948. A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. *Arch. of Biochemistry,* **16**: 131–141.

Lillie, R. D., 1948. Histopathologic Technic. The Blakiston Co., Philadelphia.

McManus, J. F. A., 1946. The histological demonstration of mucin after periodic acid. *Nature,* **158**: 202.

McManus, J. F. A., 1948. The periodic acid routine applied to the kidney. *Am. J. Pathol.,* **24**: 643–653.

Meyer, K., 1945. Mucoids and glycoproteins. Advances in Protein Chemistry. **2**: 249–275. Academic Press, Inc., N. Y.

Montagna, W., C. R. Noback and F. G. Zak, 1948. Pigment, lipids, and other substances in the glands of the external auditory meatus of man. *Am. J. Anat.,* **83**: 409–436.

Noback, C. R. and W. Montagna, 1947. Histochemical studies of the basophilia, lipase, and phosphatases in the mammalian pancreas and salivary glands. *Am. J. Anat.,* **81**: 343–367.

Rittershaus, K., 1927. Studien zur Morphologie und Biologie von *Phyllopertha horticola* L. und *Anomala aenea* Geer (Coleopt.). *Zeit. f. wissen. Biol., Abt. A.: Zeit. f. Morph. u. Oekol. der Tiere,* **8**: 271–408.

Thomas, O. L., 1947. The cytology of the neurones of *Helix aspersa*. *Quart. J. Micr. Sci.,* **88**: 445–462.

Wislocki, G. B., H. Bunting and E. W. Dempsey, 1947. Metachromasia in mammalian tissues and its relationship to mucopolysaccharides. *Amer. J. Anat.,* **81**: 1–37.

Worley, L. G., 1946. The Golgi apparatus—an interpretation of its structure and significance. *Ann. N. Y. Acad. Sci.,* **47**: 1–56.

STARVATION STUDIES WITH THE PARASITIC WASP HABROBRACON

DANIEL S. GROSCH

Department of Zoology and Entomology, N. C. State College of Agriculture and Engineering of the University of North Carolina, Raleigh, N. C.

Introduction

The present investigation on the braconid known in genetic literature as *Habrobracon juglandis* (Ashmead) suggested itself as a test of the hypothesis that the sex difference in fat cell size (Grosch, 1948c) may be interpreted on the basis of single versus double storage of reserves. That is, females are suspected to possess, in the form of ovaries, an additional and dominant storage tissue, competing in activity and mass for space in the confines of the wasp abdomen.

Furthermore if utilization of eggs occurs similar to that described for other Hymenoptera (Flanders, 1938, 1942a, 1942b) it is desirable to determine when the process begins and to what extent it proceeds, because basic techniques of important investigations on the effects of irradiation involve Habrobracon egg production (A. R. Whiting, 1949; G. A. Heidenthal, 1945).

Finally, as reference for proposed investigations on morphogenesis, it was desirable to know the size relationships of major internal structures under the normal and the most extreme conditions of the laboratory environment.

Part I: Investigations at Incubator Temperature

Because a majority of experimental rearing of Habrobracon is conducted under incubation, attention was especially directed to this environment.

Materials and methods. Wild type stock 33 provided the normal animal types used, namely impaternate males and biparental females. Since the purpose was to investigate the organism under routine laboratory conditions, no exceptional provisions for humidity control were made. Open pans (10 × 8 × 2 in.) of water were (and are) maintained on the lowest shelf of the 28 × 16 × 28 in. incubator. Temperature was maintained at 30–35° C. with maximum-minimum thermometer keeping the record. Shell vials (70 × 20 mm.), plugged with cotton (enclosed in cheesecloth), were the containers employed in stock maintainance and for all experiments. Daily examinations were made of all vials. Thus day of emergence from cocoon and day of death were obtained.

Since not even water was given to the animals under investigation, the "most extreme" type of routine laboratory environment was provided. Incidentally, results in a small scale exploratory experiment had shown average mortality to occur slightly earlier in groups provided with water, which probably is explainable by a tendancy of weakened individuals to blunder into the water and drown. This observation is consistent with Genieys' (1925) statement that mortality is very great when starving braconids are given nothing besides pure water.

For purposes of estimating the site of the major loss of substance during starvation, wasps were transected at the petiole and the weights of abdomens and weights of anterior regions were compared in freshly eclosed and in starved wasps.

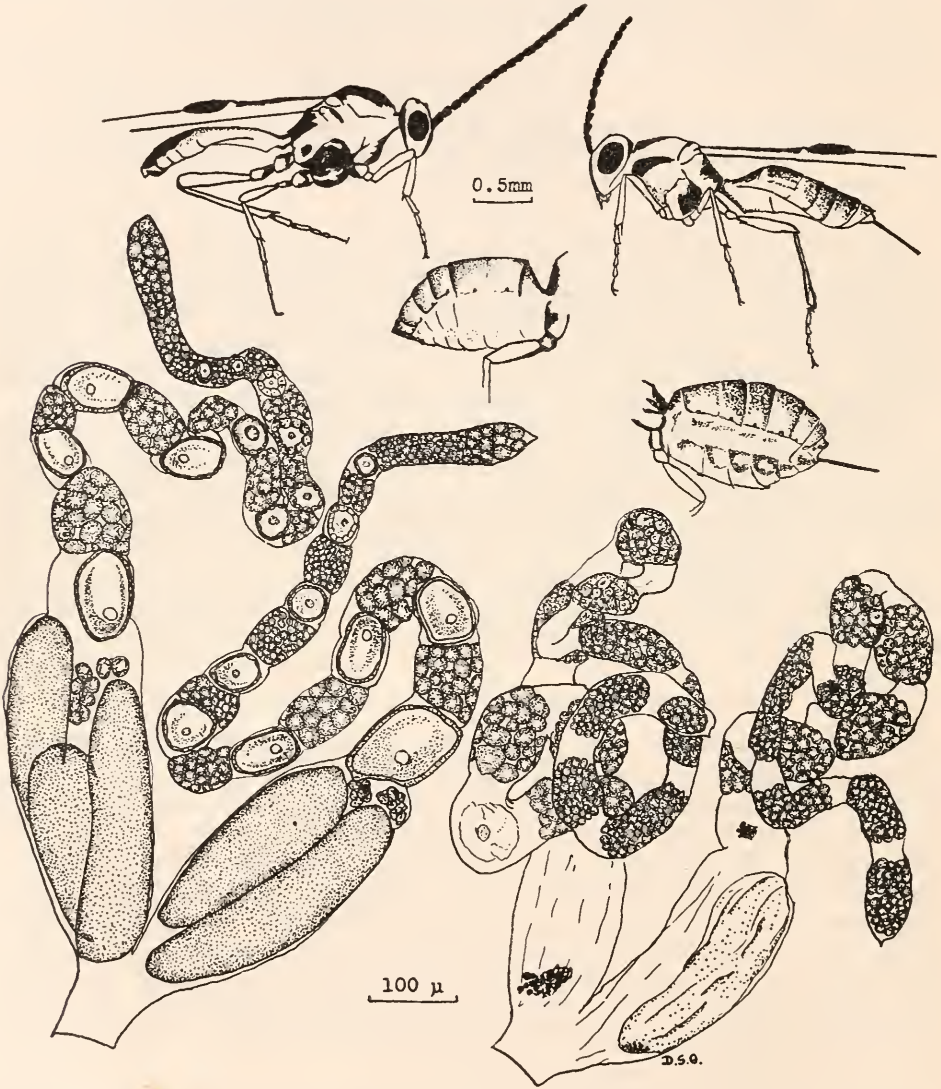


FIGURE 1. Above: Lateral views of a starved male (Left) and a starved female (Right) with abdomens of an unstarved male and female for comparison. The extreme dorsoventral flattening depicted can be seen in starved animals which are still quite active. It is interesting to note how this condition approximates the appearance of pinned museum specimens. Below: Ovarioles, each pair of which constitutes one of the two ovaries of (Left) an unstarved female recently emerged from her cocoon and (Right) a starved female dissected in what would have been the last or second last day of her life. The empty areas in the latter represent locations from which developing ova have disappeared.

For structural measurements wasps in various stages of starvation were dissected under a binocular microscope and the internal organs immediately fixed with 10 per cent formalin. This procedure was adopted because of the contrasts the fixative quickly develops in the ovaries and accessories. The preparation was then examined under a compound microscope and measurements of tissue elements taken by means of an ocular micrometer. The series of observations was continued until data was available for at least 5 wasps of each sex for each day during the starvation period.

RESULTS

Longevity. The survival time in days as given by the mean and its standard error was 6.13 ± .16 for 100 males and 6.68 ± .30 for 100 females. All animals comprising these samples were within the normal size range and no correlation between the size of animal and the survival time in days was apparent (correlation constant $r = 0$). Measured from the time of eclosion the earliest deaths occurred on day 3, oldest survivors expired on day 10.

Weight loss. Comparisons of transected wasps indicate that weight is lost chiefly from the abdomen. For 10 males of selected size (2.35 to 2.47 mm. long) the anterior parts comprising the head and thorax-with-appendages averaged (mean with its standard error) .47 ± .04 mg. at eclosion, and .45 ± .04 after starvation; while the abdomens averaged .52 ± .05 mg. at eclosion and .38 ± .06 mg. after starvation. For 10 equivalent females, anterior parts averaged .50 ± .06 mg. at eclosion and .50 ± .05 mg. after starvation, while abdomens averaged .82 ± .07 mg. at eclosion and .38 ± .06 mg. after starvation. Weight losses from the abdomen are accompanied by dorso-ventral flattening which becomes extreme in the last two days of life (see Fig. 1). In fact, in this dimension the male abdomen becomes so thin it actually approaches translucence, with shadowed areas indicating the positions of the digestive tract and gonads.

Histological changes. Presented in Table I are measurements of internal structural units in unstarved (0 hour, 0 day; at eclosion) and starved wasps (unable to stand; therefore in the last day of life). Ten animals from each class are represented by this summarization. A summary of the series of examinations of internal structures made at daily intervals is untabulated because it merely provides values intermediate between the extremes of Table I. It is evident that the perceptible changes accompanying weight loss occur in the abdominal contents and especially in the "fat" cells. When the animal is adult this type of cell is found only in the abdomen (Grosch, 1949). In reference to the fat cells there is an additional untabulated observation on the abdominal contents of starved animals. That is, it is difficult to find 100 fat cells for measurement after starvation in contrast to conditions in unstarved wasps where there are typically several hundred fat cells. On the other hand there seems to be no significant change in number of urate cells as shown by the following means and standard errors: for unstarved males 35.20 ± 4.10, for starved males 31.25 ± 4.25, for unstarved females 34.33 ± .27, for starved females 31.16 ± 3.50.

Conspicuously absent in Table I are measurements of the ovarioles. This is due to the fact that because of contortion in starved animals, length could not be determined. However, diameters, as shown in the accompanying drawing (Fig. 1), change but little in ovarioles compared from starved and unstarved females. The change which is very apparent in comparisons is the effect of a sorptive process. As seen, this has involved not only mature eggs but also developing eggs. Following these events in daily dissection, it is during the fourth day at incubator temperature that the egg contents of the ovarioles are visibly depleted. To day 3 inclusive, a total of 10 to 12 full sized eggs can be found for the four egg sacs. The contents of both the mature and the developing ova appear to be quantitatively adequate during this period. After the third day changes are visibly apparent,

TABLE I

Means and standard errors to represent dimensions in microns of internal structures of wasps 2.35 mm. to 2.47 mm. in length

Abdominal contents	Males		Females	
	Unstarved	Starved	Unstarved	Starved
Urate cell diameter	161.15± 2.64	110.22± 2.64	128.48±2 1.98	105.49± 3.19
Fat cell diameter	141.02± 1.87	89.10± 1.43	132.33± 1.43	78.21± 1.32
Crop				
Length	728.42±27.17	725.78±32.45	715.00±111.65	1005.40±119.02
Width	308.00±23.98	297.00±23.87	244.75± 44.11	294.80± 34.76
Stomach				
Length	889.13±14.41	880.00± 2.86	1031.25± 72.82	1086.25± 83.38
Width	330.00±27.50	253.00±14.74	481.25± 28.49	379.50± 33.99
Hindgut				
Length	891.00±33.60	861.63± 9.46	916.63± 15.18	941.75± 24.86
Width*	180.40± 9.57	190.63±15.62	231.00± 28.82	195.25± 4.95
Malpigh. tube				
Length	1353.00±60.39	1251.25±22.88	1375.00± 47.74	1320.00± 33.66
Width	33.00± 5.00	33.00± 1.65	33.00± 5.30	31.79± 8.80
Testis				
Length	270.60± 5.17	214.50± 4.18		
Width	184.80± 2.75	144.00± 1.75		
Accessory tube				
Length	371.25±22.77	368.50±26.07		
Width	74.25± 4.29	79.75± 4.29		
Abdominal ganglion (2nd last)				
Length	49.00± .55	49.00± .55	89.10± .55	93.50± 7.38
Width	49.00± .55	49.00± .55	91.63± 1.65	93.50± 8.69
Poison gland				
Length			770.00± 44.66	715.00± 27.50
Width			90.75± 8.36	107.25± 10.23
Reservoir				
Length			291.50± 11.88	253.00± 13.64
Width			17.05± .28	13.75± 7.26
Thoracic muscles diameters				
Dorsal long.	94.60± 3.85	96.80± 4.73	101.20± 4.73	96.80± 4.84
Alars	60.94± 1.54	59.40± .77	53.13± 1.54	50.95± 1.54
Promotors and remotors	25.19± .22	22.00± 2.09	22.00± 1.87	20.13± 1.65

* This dimension is given for the broadest part, the narrowest part in both males and females is about 77 μ in diameter.

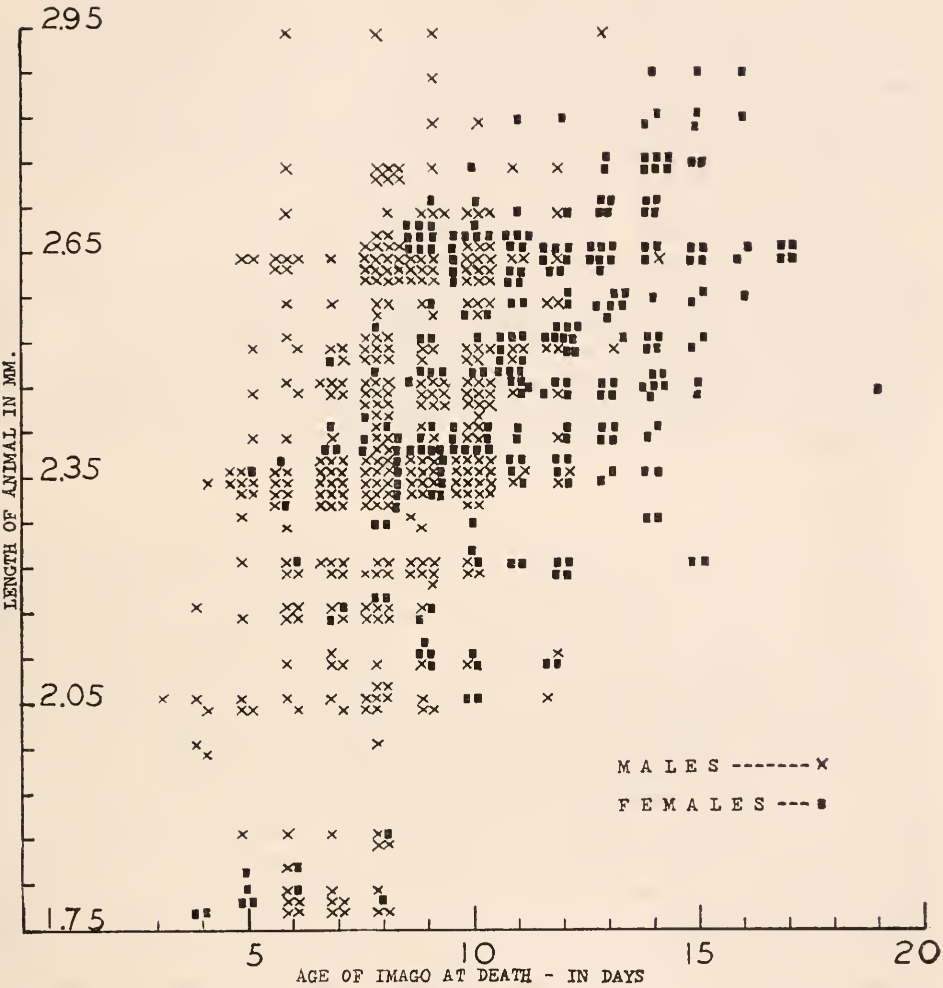


FIGURE 2. Scatter diagram showing the relative longevity of starved males and females of the sizes obtained in routine culture of Habrobracon. Notable is the tendency of females to live longer than males of equal size as reflected in the propensity of the squares to lie more to the right than the x marks.

especially in lessened opacity of egg regions. After five days of starvation no more than two eggs have been found in any dissected abdomen and after six days no full sized eggs have been found. On the other hand, there are no visible signs of sorption of developing eggs until a day or two before death (day 8 or 9 at incubator temperature). Incidentally, the relatively slight but definite change in testis size (Table I) also is not demonstrable until the last two days before death. In general these oösorption events have striking similarities to the observations on the honey bee and ants mentioned by Flanders (1942b) as being made first by Weyer.

PART II: LONGEVITY AT ROOM AND REFRIGERATOR TEMPERATURES

When it is desired to slow up development and increase the period of time between the transfers necessary for stock maintainance, common laboratory practice is to keep Habrobracon vials at room temperature. Another routine procedure is the storage of excess wasps in a refrigerator. In order to investigate an impression that females were the last survivors in vials kept under these conditions, specific observations were made on samples of wasps of known age and size.

Materials and methods. Wild type stock 33 again provided the wasps which were stored in clean shell vials. Death by causes other than starvation had been observed to accompany storage in vials containing host caterpillars, inviable pupae and other miscellaneous debris. Room storage at temperature 20–25° C. was investigated in the summertime at the Marine Biological Laboratory, Woods Hole, Massachusetts where humidity is generally high (typical relative humidity is between 70–80%). Vials stored in an electric refrigerator at 4–5° C. were placed in a closed metal container around an open wide-mouth bottle full of water.

RESULTS

Room temperature. At 20–25° C. a distinct positive correlation is evident between animal size (as represented by a measurement such as length) and longevity (as given by day of death). Correlation constants (r) were calculated as .3404 for males and .5746 for females. (Thanks is expressed to Dr. H. F. Robinson and technical assistants of the N. C. State College Dept. of Experimental Statistics for computing this pair of values.) Furthermore, the adult life of males was significantly shorter (7.98 ± .02 days) than that of females (11.27 ± .02 days) as measured by calculating mean survival time and standard error. A graphic picture of these facts is given by the scatter diagram Figure 2. An analysis also was made of these same data from the standpoint of number of individuals per vial. This comparison of viability in experiments when less than 10 (average = 5) individuals were contained per vial with those having more than 10 per vial (average = 20) shows no indication of an effect of crowding. Means for time of death of males uncrowded and crowded, are identical with the mean given above for the whole group with insignificant differences in standard errors. For females, mean survival time, in days with standard error 10.53 ± .50, was actually shorter in uncrowded vials as compared with the same computations in the larger groups, 11.63 ± .16. Differences between these means are not significant and the higher value for larger groups of females can be explained by chance inclusion of more of the longer lived wasps in the larger sample. It should be noted that even the trend is not what might be expected if adult population density is important in influencing the duration of life.

A second series of experiments at room temperature was performed with 100 females and 130 males of normal size kept in separate storage (10 per vial) from the time of their emergence as adults. No increased longevity was obtained for segregated females whether considered as a group or on the basis of extreme individuals. For segregated males a mean survival time in days of 8.38 ($s_{\bar{x}} = \pm .26$) indicates greater longevity.

Refrigerator temperature. At 4–5° C. the general principles (1) that larger animals live longer and (2) that females live longer than males of equal size were again demonstrated. Existence is greatly prolonged at lower temperatures as indicated by starved males which lived as long as 15 weeks and starved females which lived as long as 20 weeks. Using animals of normal size, 2.05 to 2.75 mm. long, the mean survival time with standard error for 193 males was 8.16 ± .17 weeks; for 109 females 9.71 ± .39 weeks.

DISCUSSION

Comparisons of measurements of internal structures indicate that under starvation conditions the female differs from the male only by one major change, oösorption. Furthermore, in spite of smaller fat cells (Grosch, 1948c) females are shown to live longer than males of equal size. This fact is interesting in view of a recent publication (Georgiana, 1950) which reports longer life of females in honey fed culture. Taken together with present results these findings suggest that the stored materials obtained by oösorption are highly efficacious in maintaining life processes. Perhaps because of egg substance females contain more stored food *value* per unit storage volume. At any rate, the present results indicate that female abdomens are heavier. In a sense these views imply a different basis of metabolism for the sexes. In normal environment the female, through her diet, appears to have a different basis for metabolic processes. She ingests host body contents and although able to lay her oldest egg without this type of meal, it seems well founded that she must continue a caterpillar diet to insure continued egg production (Henschen, 1929) and circumvent oösorption (postulated on the basis of Flanders, 1942b). On the other hand the male shows interest in no food other than honey or syrup (Grosch, 1950). Furthermore, correlative with differences in the diet are demonstrable chemical differences between the feces of the different braconid sexes (Hase, 1922). Therefore, if the internal processes of the female normally involve substances in addition to those stored in the fat body, it seems a logical conclusion that the action systems are continued in starvation and cause oösorption. Thus revealed is mass disappearance of a substance in addition to fat body in confirmation of the authors' hypothesis that structural units of the fat body do not constitute the only depository for reserves in the female system.

While difference in type of metabolism is advanced as the explanation for differences in longevity between the sexes, difference in rate of metabolism is suggested in explanation of the correlation discovered between size and longevity within a sex. It is well known that smaller animals have a higher respiratory rate and that this rate is an index of metabolic activity (Heilbrunn, 1943).

Intimately associated with size differences in Habrobracon are differences in the size of microscopic structural units including fat cells and differences in the number of eggs produced (Grosch, 1948a). These situations are traceable to conditions during the larval feeding stage, the only period in the holometabolous life cycle during which the size increases (termed growth) occur. On this basis, crowding would be more likely to affect individual longevity if it occurred during the larval stage than at any other time during the Habrobracon life cycle. Furthermore, in contrast to the behavior of Drosophila, on which classic experiments in relation of population density to longevity have been made (see Hammond, 1938, 1939), stored adult Habrobracon adopt relatively quiescent attitudes in an undisturbed vial. The same pose and position may be maintained for hours or even weeks, depending upon the temperature. These facts could explain why results in an analysis based on group size demonstrated no noticeable effect of crowding on adult longevity.

An indication that segregated males live longer than males stored with females can be explained by the fact that mating preliminaries and the act of copulation involve excited active movements by the male (Grosch, 1948b). In the absence of mating stimuli these energy expending activities would not be indulged.

The bearing of the present results upon methods of investigation involving egg production and those pertaining to morphogenesis can be summed up quite briefly. The unfed adult should be employed in experiment or examined for phenotype during its first 48 hours, an adequate period of safety unless fat cells are under consideration. In the latter event examination should be made immediately upon emergence from cocoon as emphasized in a previous publication (Grosch, 1948c). Concerning comparisons of structural size, if genetic variation is under consideration, analysis should be made within sex types because Table I indicates slight sex differences in internal abdominal structures.

Studies on changes in microscopic anatomy are not typical of past trends in entomological literature. Starvation in adult insects has been approached chiefly from the standpoint of comparative longevity of unrelated species (Jackson, 1925; Maluf, 1939; Brues, 1946) or gross physiology of the intact organism (Uvarov, 1928; Wigglesworth, 1947). On the other hand, the amount of microscopic and histochemical observation made available through malnutrition and inanition experiments with vertebrates is impressively large, as indicated by consultation of almost any volume of Biological Abstracts. However, in histological detail, situations do not seem comparable and therefore literature on vertebrate changes are not reviewed herein.

SUMMARY

1. In conditions of complete starvation, Habrobracon females live longer than males. This fact is more obvious at lower temperatures of storage.

2. There is a distinct positive correlation between size of a wasp and longevity. This is interpreted to have a basis in a lower metabolic rate in the larger organisms.

3. Weight loss is chiefly from the abdominal region which in the ultimate condition is extremely flattened dorsoventrally.

4. Histologically the greatest loss of material from both males and females is in number and size of the "fat" cells of the fat body. In females an additional loss occurs from the ovary, especially during the last stages of starvation. In wasps starved to the point of death the loss includes all the developing, as well as the mature eggs. Thus identified for the female seem to be two sources of reserve food supply. This point and attendant features are discussed from the standpoint of sex differences in structure, function and longevity.

5. Provided examination is made within the first two days, internal tissue other than the fat body should be in adequate quantitative state regardless of conditions of culture during the adult stage. It is also indicated that investigations employing ova should be started before two days after emergence from cocoons have elapsed.

LITERATURE CITED

BRUES, C. T., 1946. Insect dietary. Harvard University Press, Cambridge, Massachusetts.

FLANDERS, S. E., 1938. The effect of cold storage on the reproduction of parasitic Hymenoptera. *J. Econ. Ent.*, **31**: 633–634.

FLANDERS, S. E., 1942a. The deposition of non-viable eggs by Hymenoptera. *J. Econ. Ent.*, **35**: 283.

FLANDERS, S. E., 1942b. Oösorption and ovulation in relation to oviposition in the parasitic Hymenoptera. *Annals Ent. Soc. Am.*, **35**: 251–266.

GENIEYS, P., 1925. *Habrobracon brevicornis* Wesm. *Annals Ent. Soc. Am.*, **18**: 143–202.

GEORGIANA, M., 1950. Longevity of the parasitic wasp *Habrobracon juglandis* (Ashmead). *Am. Nat.*, **83**: 39–48.

GROSCH, D. S., 1948a. Dwarfism and differential mortality in Habrobracon. *J. Exp. Zool.*, **107**: 289–314.

GROSCH, D. S., 1948b. Experimental studies on the mating reaction of male Habrobracon. *J. Comp. and Phys. Psych.*, **41**: 188–195.

GROSCH, D. S., 1948c. The cells of the fat body in haploid and diploid types of Habrobracon. *Proc. 45th Annual Meeting N. C. Acad. of Sci., J. Elisha Mitchell Sci. Soc.*, **64**: 178.

GROSCH, D. S., 1949. Histological observations on the metamorphosis of male Habrobracon. *J. Elisha Mitchell Sci. Soc.*, **65**: 205–206.

GROSCH, D. S., 1950. Olfactometer experiments with male braconids. *Annals Ent. Soc. Am.* In press.

HAMMOND, E.C., 1938. Biological Effects of population density in lower organisms. *Quart. Rev. Biol.*, **13**: 421–439.

HAMMOND, E. C., 1939. (Same title as above.) *Quart. Rev. Biol.*, **14**: 35–39.

HASE, A., 1922. Biologie der Schlupfwespe *Habrobracon brevicornis* (Wesmael) *Arb. aus der Biol. Reichsanst. f. Land und Forstwirtshaft*, **12**: 51–78.

HEIDENTHAL, G. A., 1945. The occurrence of X-ray induced dominant lethal mutations in Habrobracon. *Genetics*, **30**: 197–205.

HEILBRUNN, L. V., 1943. An outline of general physiology. W. B. Saunders Co., Philadelphia. 2nd Ed., revised.

HENSCHEN, W., 1929. Über die Entwicklung der Geschlechtsdrüsen von Habrobracon juglandis Ashmead. *Zeitschr. Wiss. Biol. Abt. A. Morph. u. Oekol. Tiere*, **13**: 144–178.

JACKSON, C. M., 1925. Inanition and malnutrition. P. Blakiston's Son & Co., Inc., Philadelphia.

MALUF, N. S. R., 1939. The longevity of insects during complete inanition. *Am. Nat.*, **73**: 280–285.

UVAROV, B. P., 1928. Insect nutrition and metabolism. A summary of the literature. *Trans. Ent. Soc. London, 1928*, **Part II**: 255–343.

WHITING, A. R., 1949. Androgenesis, a differentiator of cytoplasmic injury induced by X-rays in Habrobracon eggs. *Biol. Bull.*, **97**: 210–220.

WIGGLESWORTH, V. B., 1947. The principles of insect physiology. Methuen & Co. Ltd., London.

A TWO-TENTACLED, COMMENSAL HYDROID FROM CALIFORNIA (LIMNOMEDUSAE, PROBOSCIDACTYLA)

CADET HAND[1] AND JOHN R. HENDRICKSON[2]

INTRODUCTION

The new hydroid, *Proboscidactyla sp.*, which is the subject of this paper, was found living upon the tubes of a sabellid polychaete, *Pseudopotamilla ocellata* Moore, at Pacific Grove, California. The authors were able to collect and observe colonies during the summer of 1949 at the Hopkins Marine Station, and living colonies secured in September of that year were brought to Berkeley, California, for photography and further study.

The genus Proboscidactyla was established by Brandt (1834) for a new medusa, *P. flavicirrata*. Somewhat later Forbes (1846) described another medusa, *Willsia stellata*, for which he established the family Willsiadae. The generic name Willsia was changed to Willia by A. Agassiz (1862) because of an orthographical error on Forbes' part, and the family name then became Williidae. This family later came to include both Willia and Proboscidactyla. The Williidae were monographed by Browne (1904), who included six species of Proboscidactyla and two of Willia.

One of the most recent considerations of the family is that of Kramp (1940), who reviews the history of the Williidae and places this family with the families of Olindiidae and Moerisiidae in the suborder Limnomedusae, a group equal in rank to the Anthomedusae and Leptomedusae. Kramp defines the Limnomedusae as follows (p. 506):

"Hydrozoa with alternation of generations. The asexual generation is a sessile polyp with ability of asexual reproduction, with or without tentacles; the endoderm of the tentacles, when present, is in direct connection with that of the gastral cavity. The sexual generation is a velar medusa with hollow tentacles; gonads either on the walls of the stomach with perradial continuations along the radial canals, or on the radial canals alone; in the latter the umbrella-margin is provided with internal statocysts with an endodermal axis."

The suborder Limnomedusae was established by Kramp to include certain rather aberrant hydromedusans. This group seems to stem from both the Anthomedusae and Leptomedusae and may be polyphyletic.

Kramp (1940) also defines the family Williidae as follows (p. 509):

"Limnomedusae without statocysts; stomach with perradial lobes extending along the proximal parts of the radial canals; gonads surrounding the stomach and its lobes; radial canals branched; tentacular bulbs without ocelli."

The two genera of the Williidae were initially separated on the basis of the number of primary radial canals; Willia having six and Proboscidactyla four.

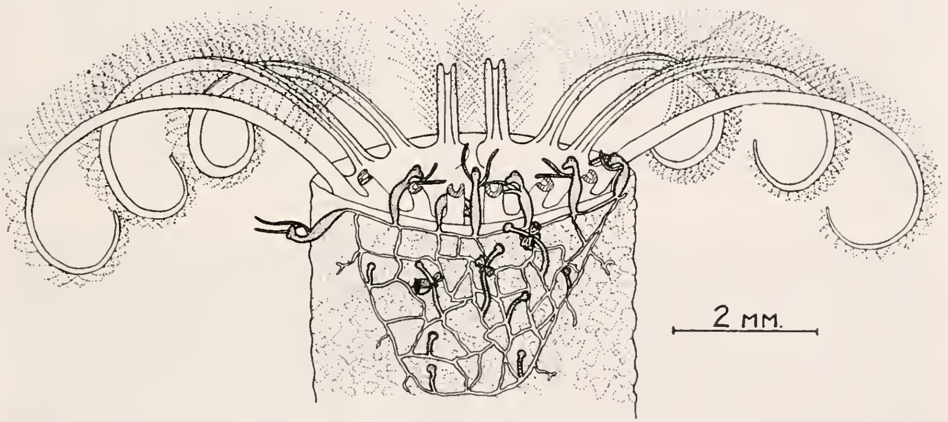
---

[1] Department of Zoology, Mills College, Oakland, California and Department of Zoology, University of California, Berkeley.

[2] Department of Zoology, University of California, Berkeley.

Some interesting information on the relationship of the above two genera was brought forth in a study of *Willia mutabilis* by Browne and Kramp (1939). In studying over 100 specimens they found 22 of these medusae with six primary radial canals and 41 with eight apparently primary radial canals. In the latter group, however, it was found that of the eight canals only four were truly primary, the other four arising not directly from the stomach but as branches of the primary canals, and thus being secondary in origin. Browne and Kramp also found specimens with 5, 7, 9, 10 and 11 apparently primary radial canals. The above data were taken by Browne and Kramp to mean that "The limit between the two genera Willia and Proboscidactyla is not so sharp as formerly supposed . . ." (p. 310), and that the species under study ". . . even tends to efface the limits of the two genera" (p. 302).

Until 1941 only two hydroids had been associated with the Williidae, and only in one case (*Willia stellata*) had the hydroid been shown to give rise to a specific medusa of this family. This latter hydroid was first described as *Lar sabellarum* by Gosse (1857), who found it growing upon the tube of *Sabella vesiculosa* which he was keeping in his aquarium. According to E. T. Browne in "Plymouth Marine Fauna," 1931, Lar occurs on *Potamilla torelli*.



TEXT FIGURE 1. A colony of *Proboscidactyla* sp. in place upon a tube of *Pseudopotamilla ocellata*. Several of the worm's tentacles have been cut away, and the number of tentacles shown on the worm is reduced from the natural condition.

The generic name Lar was derived from the Roman "Lares" (household gods), and is particularly apt when one considers the oddly human appearance of this hydroid. Gosse noted that this hydroid had ". . . a most ludicrously-close resemblance to the human figure," and described its activities as follows (p. 113): "The head lobe moved to and fro on the neck; the body swayed from side to side, but still more vigorously backward and forward, frequently bending into an arch in either direction; while the long arms were widely expanded, tossing widely upward, and then waved downward, as if to mimic the actions of the most tumultuous human passion." Plate I, Figure 1 reproduces Gosse's figure of a colony of Lar upon the extremity of a tube of *Sabella vesiculosa*; as will be seeen later, this hydroid closely resembles the form to be described in this paper.

The production of medusae by the hydroid *Lar sabellarum* was described by Hincks (1872), who noted that the medusae produced were similar to *Willia stellata*. Browne (1896), in a thorough study of the growth of *Willia stellata*, was able to show clearly that the medusa of *Lar sabellarum* actually was *Willia stellata* and so *Lar sabellarum* became a synonym of *Willia stellata*. The family Laridae, which had been established for the hydroid Lar was thus invalidated, although Fraser (1918, 1946) continued to use Gosse's Lar and Laridae.

The second known hydroid of the Williidae was described by Mereschkowsky (1877) as *Monobrachium parasitum*, for which he created a new family, Monobrachiidae. This hydroid differs from Lar in that it possesses but one tentacle, produces medusae with four primary radial canals and is found around the siphons on the shells of small subtidal bivalved molluscs. This hydroid is thought to be a Proboscidactyla because of the branching radial canals of its medusa, although it has not been associated with any specific adult medusa. Again Fraser (1918, 1946) recognizes the family name Monobrachiidae rather than the Williidae, of which he makes no mention.

In 1941, Uchida and Okuda described a new "Lar" which they found living on *Potamilla myriops* in Akkeshi Bay, Hokkaido, Japan. This hydroid was shown by them to be that of the medusa *Proboscidactyla flavicirrata*. This meant that the genera Willia and Proboscidactyla were both characterized by similar hydroids. These authors found, further, that the young medusae of *P. flavicirrata* had four, five or six radial canals, spanning the difference between Willia and Proboscidactyla. On the basis of the above evidence, plus the data of Browne and Kramp (1939) on the variability of *Willia mutabilis*, Uchida and Okuda united the two genera, adopting the name Proboscidactyla because of priority. Although not stated by Uchida and Okuda, the family name should accordingly be changed from Williidae to Proboscidactylidae.

DESCRIPTION

a. *Habitat*

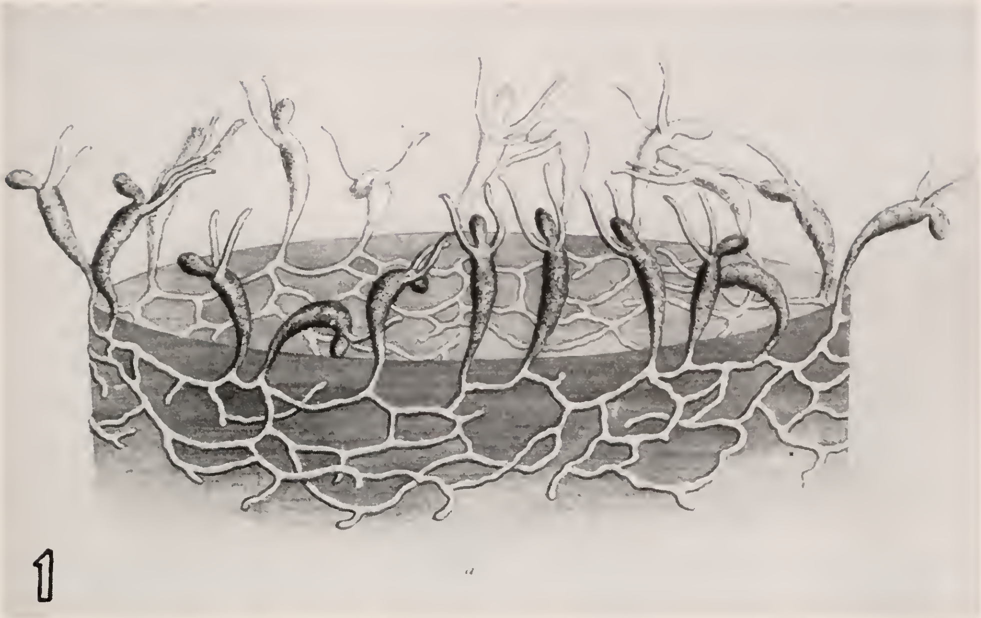
The new Californian hydroid is found growing upon the tubes of a sabellid polychaete, *Pseudopotamilla ocellata* Moore, and has been collected from several stations along the Monterey Penninsula from Carmel Cove to Point Pinos. The sabellid seems to be restricted to rocky areas of the intertidal zone around the mean lower low water line, where it lives in crevices and under or between rocks. The tube of the worm is of a parchment-like substance, richly embedded with sand grains, although the terminal free portion is frequently less sandy. The sabellid is free to turn within its tube. When the worm withdraws, the terminal portion

PLATE I

FIGURE 1. A reproduction of Gosse's (1857) figure of *Lar sabellarum* on the extremity of a tube of *Sabella vesiculosa*.

FIGURE 2. A living colony of *Proboscidactyla* sp. on a tube of *Pseudopotamilla ocellata* (ca. 25 ×).

Plate I



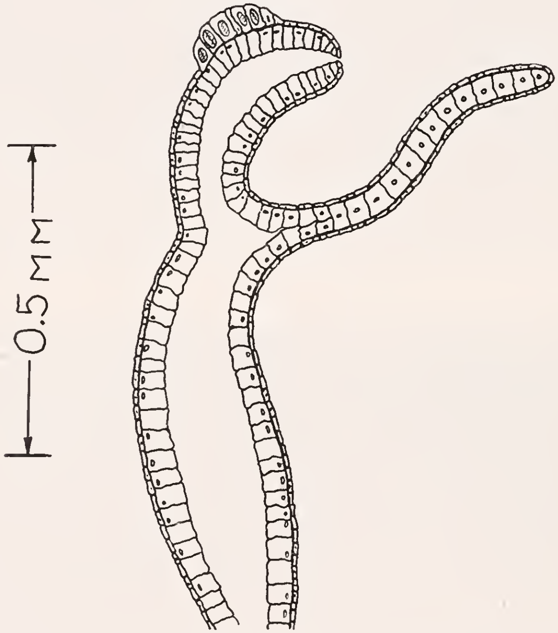
1

2

of the tube flattens and one edge folds over the other, thus covering many of the zooids at the edge of the tube.

b. *The hydroid colony*

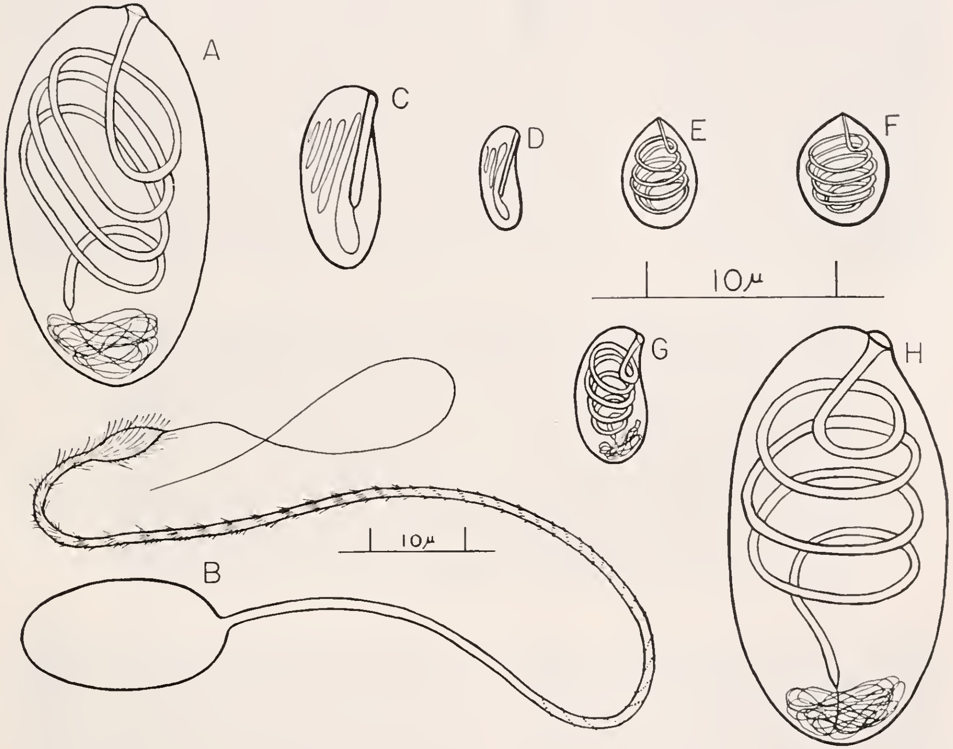
The colony consists of two, or perhaps three kinds of individuals: gastrozooids, gonozooids and possibly dactylozooids. The gastrozooids are found only at the rim of the worm tube. In some colonies the gastrozooids are evenly distributed around the aperture of the tube, while in others they are clumped on the side of the tube which will fold under when the worm retires. The gonozooids are located on the body of the tube, apparently never on the rim. They are attached to the gastrozooids and to each other by an anastomosing system of naked hydrorhizae, whereas the hydrorhizae of typical Anthomedusae always have a perisarc. The gonozooids, at the seasons we observed them, usually bore from one to four medusa buds in various stages of development. The whole colony may extend from the rim of the tube to a distance of 1.5 cm. down its length. At the edges of the colony farthest from the gastrozooids were found very short capitate zooids which may have been dactylozooids, although their nematocysts are identical to the gonozooids, as is their appearance, except that they are smaller and lack medusa buds. Text Figure 1 represents a colony in place upon a sabellid tube. Several of the worm's tentacles are shown as cut away to allow a better view of the colony, and the number of tentacles represented on the worm is considerably reduced from the actual condition.



TEXT FIGURE 2. Diagrammatic longitudinal section through a gastrozooid of *Proboscidactyla* sp.

c. *The gastrozooids*

The gastrozooids are about 0.8 mm. tall, usually less than 0.1 mm. in diameter, and possess two tentacles located at about ¾ the distance from base to top. These tentacles arise from a common area but separate immediately upon becoming distinct from the body. They are highly extensible and may stretch to about 2 mm. in length. The tentacles are solid and their endoderm is in contact with the endoderm of the body. Text Figure 2 is a diagram of a longitudinal section through a gastrozooid showing the relationship of the endoderm of the tentacles and body. The "head" is separated from the "body" by a distinct "neck" and is capped by an oval battery of nematocysts. The "head" and "neck" are curved, making an arc of 90°, and the mouth is terminal, facing the side of the "body" on which the tentacles are located. The "body" is spindle-shaped, being largest at its midpoint and tapering at its lower end, decreasing to the size of the hydrorhizae, or about 0.03 mm. Plate 2, Figures 3 and 4, are photographs of living isolated gastrozooids. The gastrozooids are oriented around the top of the tube so that



TEXT FIGURE 3. Nematocysts of *Proboscidactyla* sp.: a–e, of the gastrozooid; f–h, of the medusa. (a. unexploded macrobasic eurytele; b. exploded macrobasic eurytele; c. large microbasic eurytele; d. small microbasic eurytele; e. desmoneme; f. desmoneme; g. small macrobasic eurytele; h. large macrobasic eurytele.)

they always face the tube opening. Plate 1, Figure 2, shows a living colony on a tube from which the worm has been removed.

d. *Nematocysts of the gastrozooid*

The types and approximate sizes of the nematocysts of the gastrozooids are given below. The measurements reported for these nematocysts and for the medusa are for discharged capsules. The gastrozooids possess (see Text Fig. 3a–e):

1. Desmonemes: 8 × 4 μ.
2. Telotrichous macrobasic euryteles: 22 × 11 μ.
3. Large microbasic euryteles: 11 × 4 μ.
4. Small microbasic euryteles: 6 × 2 μ.

The macrobasic euryteles occur only in the nematocyst pad on the head.

The nematocysts of the gastrozooid of *Willia stellata* have been described by Russell (1938), who found nematocysts similar in size and type to those above, with the exception of the macrobasic euryteles. Russell did not find these, but found instead macrobasic mastigophores which were armed throughout the length of the hampe. In regard to the mastigophores he says (p. 145), "In some specimens treated with acetic acid or distilled water the distal end of the hampe swelled into an ampulla, like that in the macrobasic euryteles of Zanclea." Russell's figure of such a capsule seems to illustrate a eurytele which appears identical in type to the macrobasic euryteles (Text Fig. 3a, b) found in the Californian Proboscidactyla. In making our preparations of nematocysts we have used sea water and have never found mastigophores; we suggest that the macrobasic mastigophore reported by Russell is really a macrobasic eurytele. This would make the nematocysts of the gastrozooid of *Willia stellata* identical in type to those of the Californian Proboscidactyla. Further, macrobasic mastigophores are not known for gymnoblastic hydroids other than as reported by Russell above.

e. *The gonozooids*

Mouthless gonozooids (Text Fig. 4 and Plate 2, Fig. 5) occur on the sides of the worm tubes, attached to one another and to the gastrozooids by naked hydrorhizae. They may occasionally be 1 mm. tall but frequently are shorter. The gonozooids are capitate, the distal sphere being heavily studded with nematocysts. The medusae are borne at about the middle of the gonozooid, and a maximum of four medusa buds may be present. These buds apparently arise successively, so that

PLATE II

FIGURE 3. Isolated living gastrozooids of *Proboscidactyla* sp. removed from the colony shown in Plate I, Figure 2 (ca. 50 ×).

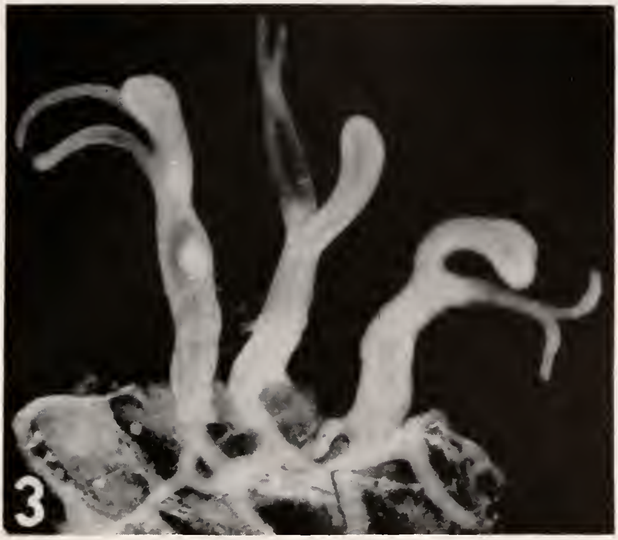
FIGURE 4. A single living gastrozooid of *Proboscidactyla* sp. removed from the colony shown in Plate I, Figure 2 (ca. 50 ×).

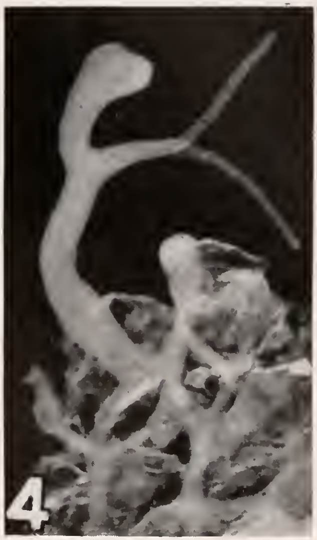
FIGURE 5. A living gonozooid of *Proboscidactyla* sp. bearing several medusa buds (ca. 35 ×).

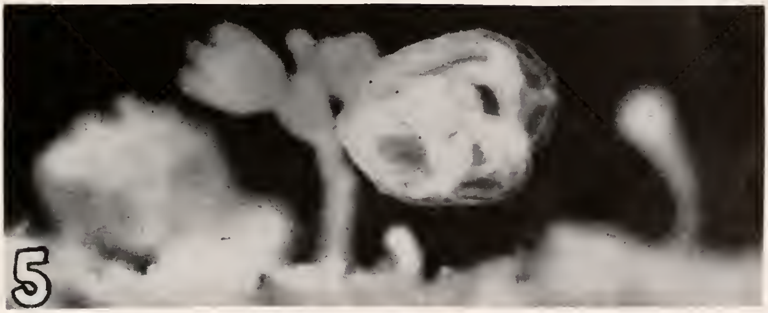
FIGURE 6. A living pentamerous medusa of *Proboscidactyla* sp., oral view, one day after release (ca. 50 ×).

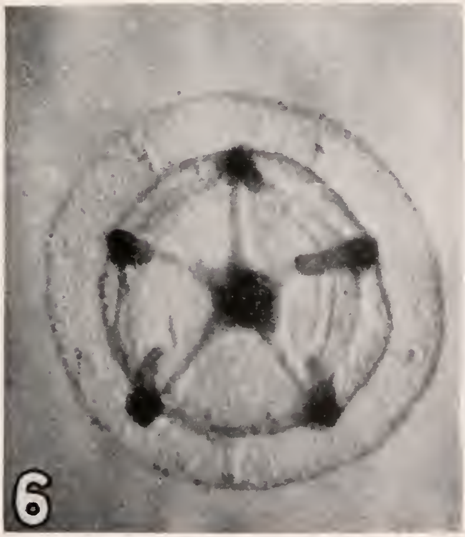
FIGURE 7. A living tetramerous medusa of *Proboscidactyla* sp., one day after release (ca. 50 ×).

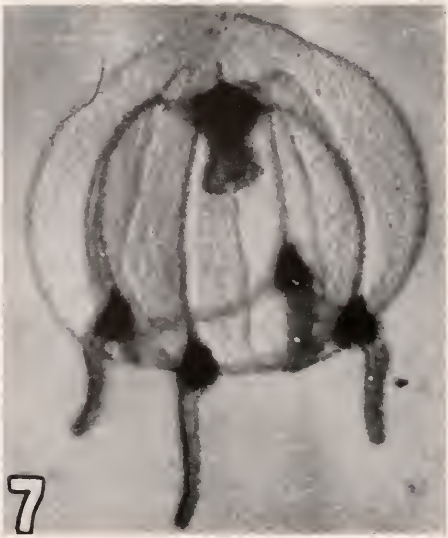
Plate II


3


4


5


6

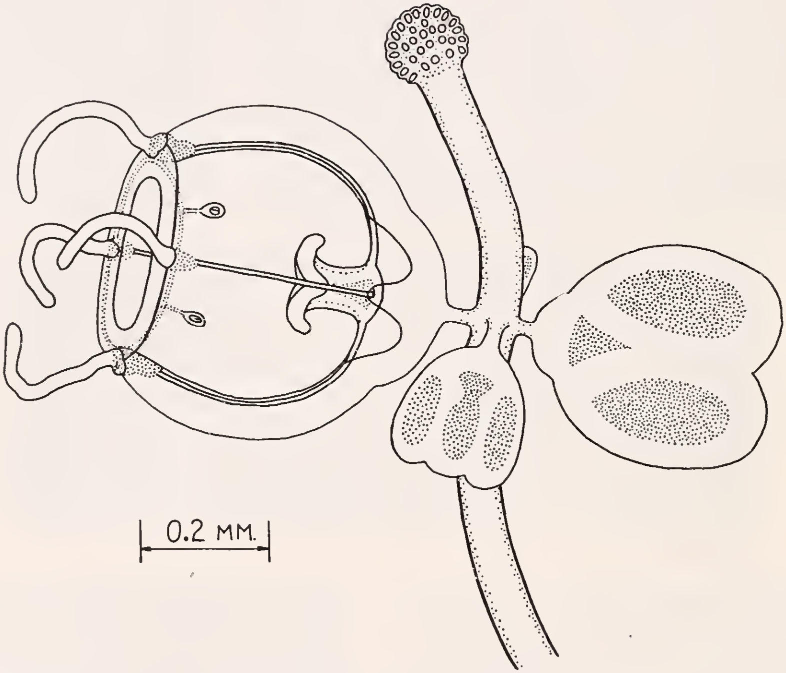

7

if four are present there are always a large one and three successively smaller ones arranged as in Text Fig. 3. In our specimens the larger medusa buds were seen to pulsate vigorously for a day or two before they were released. Many of the gonozooids possessed 1, 2 or 3 immature buds with no pulsating medusae.

The nematocysts of the gonozooids are the same as those of the gastrozooids except that no small microbasic euryteles are present.

f. *The medusae*

In the course of this study we have seen 25 released medusae. With a single exception, each medusa possessed four primary radial canals, the exception having five. Plate 2, Figure 6, is a photograph of the living, one day old, pentamerous medusa, and Plate 2, Figure 7 illustrates one of the tetramerous specimens. The medusa possessing five canals was noted before its release and occurred on a gonozooid which also bore normal tetramerous buds. Text Figure 5 shows a medusa as it appears shortly after release, at which time the medusae are about 1.3 mm. tall and 1.0 mm. in diameter. There are four interradial cnidothylacies (nematocyst sacs) which contain large and small macrobasic euryteles, and four tentacles possessing desmonemes and small macrobasic euryteles. The medusa with five radial canals had five tentacles. In no case in the immature medusae which we observed did we find any sign of branching of the radial canals. The ring



Text Figure 4. A gonozooid of *Proboscidactyla* sp. bearing medusa buds in different stages of development.

canal is represented by a solid cord, to which each of the interradial nematocyst sacs is attached by a strand of endodermal cells. The tentacles are hollow, inserted into the bell by means of large orange-brown pigmented endodermal bulbs, and are marked by nearly regular circular ridges which contain nematocysts. The stomach is four-lobed, each lobe leading into a radial canal. The manubrium is also four-lobed and hangs free under the bell. No statocysts or ocelli have been observed.

All medusae examined were very immature, and neither sections nor microscopic examination of the whole medusa showed any signs of the location of the gonads.

g. *Nematocysts of the medusa* (Text Fig. 3f–h)

1. Desmonemes: 6 × 5 μ.
2. Large telotrichous macrobasic euryteles: 25 × 11 μ.
3. Small telotrichous macrobasic euryteles: 8 × 4 μ.

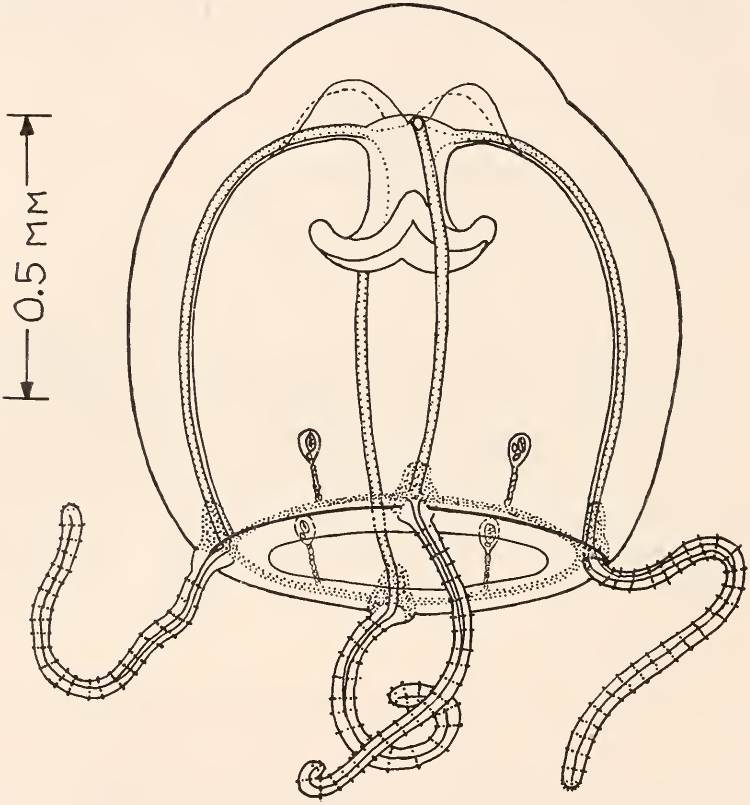
No microbasic euryteles were found in the medusae.

Relationships Between Hydroid and Sabellid

Upon observing the feeding currents of Pseudopotamilla, one finds strong currents moving into the tentacular crown from a posterior direction and passing out anteriorly. There are also special collecting currents which run along the pinnules and down the axis of each branchial filament toward the mouth. Food which is trapped by the filtering mechanism of the crown is carried along these paths onto the palps and lips, from which it is transferred to the mouth. These observations agree with those of Nicol (1930) on *Sabella pavonina*. Nicol also described rejection currents on the lips which carry rejected material to the bases of the branchial filaments and then to the palps, from which the material is discarded. These rejection currents were not observed in Pseudopotamilla, although they presumably occur.

The living gastrozooids of Proboscidactyla are in continual motion. These movements, which have also been noted as characteristic of *Willia stellata* (Gosse, 1859; Brown, 1915), seem to be feeding motions. The gastrozooid may lean far backward to catch food coming to the worm in the incoming current, in one observed case snaring a large piece of detritus which it promptly transferred to its mouth. The gastrozooids also lean forward, sometimes embracing the bases of the branchial filaments with their tentacles; in other cases they lean far into the aperture of the tube and quickly move their tentacles over the surfaces of the lips and palps of the worm. In the cases where the gastrozooids embraced the branchial filaments they were observed actually to remove particles of food, which the worm had collected, from the groove in the axis, which food they promptly devoured. In other cases they searched the surfaces of the lips and palps, and seemed to be gathering food from the currents there. The gastrozooids swing their bodies and tentacles in movements which apparently cover every available area in search of food particles. The rejection currents also presumably carry food to Proboscidactyla, which is in an ideal position to reach into these currents; certainly the area where the rejection currents occur are well covered by the gropings of the tentacles. Thus this hydroid, living as it does on a host which possesses strong ciliary feeding currents, is able to utilize these currents to supply food for itself.

Many individual gastrozooids were observed whose bodies were distended with food particles as in Plate 2, Figure 3. When such individuals were sectioned, the contained material appeared to be eggs in various stages of digestion. Uchida and Okuda (1941) reported that *P. flavicirrata* readily ate the eggs of its host, *Potamilla myriops*, as the worm spawned. We do not know the source of the eggs contained by the Californian Proboscidactyla; perhaps they were the eggs of the host or perhaps they were foreign.



TEXT FIGURE 5. A medusa of *Proboscidactyla* sp. shortly after release.

The two-tentacled condition of Proboscidactyla seems to fit it well for its way of life as a commensal. These tentacles are unusually maneuverable and active for a hydroid, and their location on the gastrozooid enables it easily to lean between the branchial filaments, whereas a larger number of tentacles might be a handicap by becoming entangled with the worm. Also the great length to which the tentacles can extend allows the hydroid to cover more territory and thus increases the amount of food it can capture. We have never found this hydroid associated with any animal except *Pseudopotamilla occellata*.

Discussion

The hydroid described in this paper is similar to those of *Proboscidactyla stellata* and *P. flavicirrata*, but differs from them in that the Californian Proboscidactyla apparently never has gonozooids arising from the gastrozooids. Uchida and Okuda (1941, p. 435) report for *P. flavicirrata*, "The blastostyles are sometimes branched off from the gastrozooids," and Brown (1915, p. 167) says of *P. stellata*, that in the colonies he observed one gonozooid arose ". . . from the base of each polyp at the back," although Hincks (1872) figures gastrozooids and gonozooids arising separately in that species. In all other features of their morphology these three hydroids apparently are remarkably similar. We have found no gastrozooids bearing more than two tentacles, although Brown (1915) and Uchida and Okuda (1941) report occasional individuals with three or more tentacles.

The habitats of all threee hydroids also show remarkable similarities, *P. stellata* living on *Potamilla torelli*, *P. flavicirata* on *Potamilla myriops* and the Californian Proboscidactyla on *Pseudopotamilla ocellata*.

As for the immature medusae described by us, there is little or no way to distinguish them with certainty from other species of Proboscidactyla. The young medusae of *P. stellata* are predominantly hexamerous, although an occasional pentamerous one is found, while those of *P. flavicirrata* vary from tetramerous to hexamerous. *P. mutabilis* is also highly variable. In the Californian form we have found mostly tetramerous medusae as mentioned above. There is an important difference, however, between the Californian species and *P. flavicirrata*. Uchida and Okuda (1941) described the presence of minute eye-flecks on the abaxial faces of the tentacle bases in immature *P. flavicirrata*. These eye-flecks become obscure or are lost in the adult. We have found no such eye-flecks in the Californian material, nor have such flecks been reported for any other member of the family. The presence of these eye-flecks in *P. flavicirrata* necessitates a change in the family definition, which we suggest should read as follows:

Proboscidactylidae:

> Limnomedusae without statocysts; stomach with perradial lobes extending along the proximal parts of the radial canals; gonads surrounding the stomach and its lobes; radial canals branched in the adult; interradial cnidothylacies present; tentacular bulbs usually without ocelli except in young stages of certain species.

Just where Mereschkowsky's hydroid, *Monobrachium parasitum*, fits into this group is not known. Unfortunately, the descriptions of the medusa of Monobrachium are very incomplete, and beyond the fact that the radial canals branch little is known of it. If Monobrachium is a true Proboscidactyla, then we would have a rather striking example of divergent evolution among the hydroids of the genus, some of them being specialized for life as commensals with sabellids and others specialized to live with clams. The hydroid of Monobrachium differs from that of Proboscidactyla in lacking the pronounced "neck" and in possessing but one tentacle. Also the medusae in Monobrachium arise directly from the hydrorhizae rather than from gonozooids so it is possible that Monobrachium will prove not to be a Proboscidactyla and may not even belong to the Proboscidactylidae. But it will be interesting, should Monobrachium be shown to be a true Proboscidactyla, to see whether this rather unusual hydroid also has a medusa which is divergent, or whether the medusa is of the normal pattern while the hydroid generation alone has diverged.

The specific identification of our Californian hydroid must await further information. Only one Proboscidactyla, *P. occidentalis*, a medusa, has ever been reported from Californian waters. This was described by Fewkes (1889) from Santa Cruz Island. There is nothing in Fewkes' description which enables us to identify our form with his. This is not unusual when one considers the generalized characters of the immature medusae of this group; indeed, our form could be identified with almost any other Proboscidactyla as readily as it could be with Fewkes' species. The closeness of the localities (Monterey Penninsula and Santa Cruz Island) suggests that the Californian Proboscidactyla described by us may be identical with *P. occidentalis*, but until further stages in the development of our form are known this identification must remain speculative.

There still remain species of Proboscidactyla for which no hydroid stages are known, among which one American form merits attention. This is *Proboscidactyla* (= *Willia*) *ornata* (McCrady), a medusa which has been reported from the Woods Hole region. Hargitt (1904, p. 40) reports it as, "More or less common at irregular intervals. Occasionally taken in numbers in the Eel Pond and in the tow of the harbor." There are also at least two sabellids (*Parasabella microphthalma* (Verrill) and *Pseudopotamilla oculifera* (Leidy)) in the Woods Hole area which would seem to be possible hosts for the "Lar" stage of *P. ornata*. The authors hope that the attention of biologists at Woods Hole may be attracted to this problem, and that the hydroid of *P. ornata* will not long remain unknown.

SUMMARY

1. The history of the Proboscidactylidae is summarized and changes are suggested in the definition of the family.

2. A new Californian hydroid is described as *Proboscidactyla* sp. This hydroid may be that of *Proboscidactyla occidentalis* Fewkes.

3. The new hydroid lives as a commensal upon the tubes of a sabellid polychaete, *Pseudopotamilla ocellata* Moore.

4. The commensal relations of the hydroid to the host worm are described, especially the utilization by the hydroid of the feeding currents produced by the worm.

5. The relationship of Proboscidactyla to *Monobrachium parasitum* is discussed.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Dr. C. M. Yonge, University of Glasgow and Visiting Professor, University of California, 1949, Mr. F. S. Russell, Director of the Plymouth Laboratory, and Dr. R. I. Smith, University of California, for their suggestions and advice, and to the director and staff of the Hopkins Marine Station for considerations shown. We also wish to thank Mr. D. P. Abbott, University of California, for the photographs of the living medusae, and Dr. Olga Hartman, Hancock Foundation, University of Southern California, for the identification of the sabellid.

LITERATURE CITED

Agassiz, A., 1862. The acalephan fauna of the southern coast of Massachusetts (Buzzards Bay). *Proc. Boston Soc. Nat. Hist.*, **8**: 224–226.

Brandt, J. T., 1834. Prodromus Descriptionis Animalium ab H. Mertensio in orbis terrarum circumnavigatione observatorum. *Fasc. I. in Rec. Act. Seances, publiques Acad. Imp. Sci. St. Petersbourg*, 1833–1834.

Browne, E. T., 1896. On British hydroids and medusae. *Proc. Zool. Soc. London.* 1896, pp. 459–500, pls. 16, 17.

——, 1904. Hydromedusae, with a revision of the Williadae and Pentasidae. In Gardiner, J. S., *Fauna and Geography of the Maldive Archipeligo.* **Vol. II**, pt. 3: 722–749, pls. 54–57.

——, and P. L. Kramp, 1939. Hydromedusae from the Falkland Islands. *Discovery Reports*, **18**: 265–322, pls. 14–19.

Brown, N. E., 1915. A posturing polyp, *Lar sabellarum. Jour. Micrology*, **1**: 164–168, 1 pl.

Fewkes, J. W., 1889. New Invertebrata from the coast of California. *Bull. Essex Institute*, **21**: 99–146.

Forbes, E., 1846. On the pulmograde medusae of the British Seas. *Ann. Mag Nat. Hist.*, **18**: 284–287; also in *Rept. Brit. Assoc. Adv. Sci., Trans.*, **16**: 81–85.

Fraser, C. M., 1918. Monobrachium parasitum and other West Coast hydroids. *Trans. Roy. Soc. Canada.* 3rd Series, **12** (Sect. IV): 131–138, 2 pls.

——, 1946. Distribution and relationship in American hydroids. Toronto.

Gosse, P. H., 1857. On a new form of corynoid polypes. *Trans. Linnean Soc.*, **22**: 113–116, 1 pl.

Hargitt, C. W., 1904. The medusae of the Woods Hole region. *Bull. Bur. Fish.*, **24**: 21–79, 7 col. pls. and many text figs.

Hincks, T., 1872. On the hydroid *Lar sabellarum* Gosse, and its reproduction. *Ann. Mag. Nat. Hist.*, 4th Series, **10**: 313–317, 1 pl.

Kramp, P. L., 1940. Occasional notes on Coelenterata III. *Vidensk. Medd. Dansk. naturh. Foren.*, Copenhagen. **103**: 503–516, text figs.

Marine Biological Association, 1931. Plymouth Marine Fauna, 2nd ed.

Mereschkowsky, C., 1877. On a new genus of hydroids from the White Sea, with a short description of other new hydroids. *Ann. Mag. Nat. Hist.*, 4th Series, **20**: 220–229, 2 pls.

Nicol, E. A. T., 1930. The feeding mechanism, formation of the tube, and physiology of digestion in *Sabella pavonina. Trans. Roy. Soc. Edin.*, **56** (3): 537–598, 2 pls., 21 text figs., 7 graphs.

Russell, F. S., 1938. On the nematocysts of hydromedusae. *Jour. Mar. Biol. Assoc., U. K.*, **23**: 145–165, text figs.

Uchida, T. and S. Okuda, 1941. The hydroid Lar and the medusa Proboscidactyla. *Jour. Fac. Sci. Hokkaido Imp. Univ.*, Series VI, **7** (4): 431–440, 11 text figs.

THE DEVELOPMENT OF RECIPROCAL ANDROGENETIC FROG HYBRIDS [1]

ANNA-BETTY CLARK MOORE

Department of Zoology, Columbia University, New York City

The role of the cytoplasm in development has long been of interest to embryologists. As early as 1898, Driesch described echinoderm hybrids as purely maternal and concluded that early development is determined by the egg cytoplasm. Conklin (1908) wrote that early development, which includes polarity, symmetry, type of cleavage, relative positions and proportions of future organs, is predetermined in the cytoplasm of the egg. Conklin pointed out that these differentiations arise during ovarian history as a result of the inter-action of the nucleus and the cytoplasm, and thus both play a part in the predetermination of the cytoplasm. These conclusions were based by Conklin on his study of Ascidian eggs (1905) and Wilson's studies of Dentalium and Patella (1904).

One approach to the question of the role of the cytoplasm in development has been the study of amphibian embryos obtained by fertilizing, with foreign sperm, eggs whose maternal chromosomes have been inactivated or removed. P. Hertwig (1916) was one of the earliest workers in this field but it was Baltzer (1920) who initiated the great bulk of this work, using androgenetic Triturus hybrids. These developmental studies have demonstrated the major role of the nucleus in development but the part played by the cytoplasm has not been clarified. I believe that one reason for the failure to demonstrate the cytoplasmic role may be due to the fact that a comparison of reciprocal androgenetic hybrids and androgenetic controls has not been made. Differences in development which might appear in such a comparison may well be ascribed to the action of the cytoplasm. Suitable for such a study are the two morphologically and physiologically distinct species, *Rana pipiens* Schreber and *Rana palustris* Le Conte. Moore (1941) has shown that reciprocal hybrids of these species develop normally and metamorphose, with the hybrids intermediate in appearance between the two species.

The development of the reciprocal androgenetic hybrids between these two frog species will be described in this paper. Their development will be compared with each other and with their androgenetic controls, with the hope that some information may be obtained on the role of the cytoplasm in early development.

I wish to thank Professor L. G. Barth for his interest and guidance in this work.

MATERIALS AND METHODS

Eggs and sperm of *R. pipiens* and *R. palustris* were used in these experiments. The eggs were obtained by either pituitary induced ovulation or from females collected in the breeding season with the eggs in the oviducts. Sperm were obtained by cutting up the testes in a small quantity of spring water. Approximately 20 minutes after fertilization the eggs were examined to see if the region where the second polar body comes off was visible. This region, which is called the "Keimpunkt" or "Eispek" by the German authors, is a very small, round, dark indentation on the surface of the animal pole and marks the position of the second maturation division spindle (Fig. 1). Using the method of Porter (1939), the maternal chromosomes were removed by inserting the tip of a sturdy glass needle under the Eispek and lifting the second maturation division spindle out in a small exovate. The eggs subsequently cleaved with only the paternal chromosomes. This method of removal of the maternal chromosomes is successful 97 per cent of the time. The removal of a small exovate does not injure the egg nor alter its later development. This was tested by removing a small amount of protoplasm a short distance from the Eispek. No effect on subsequent development was noted. At the early blastula stage the androgenetic embryos, marked by the small exovate, were separated from the diploid embryos. Only blastulae in which the operation had been well performed were maintained. Approximately equal numbers of diploid blastulae were kept as controls. The embryos were raised in a 12° C. cold room.

---

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

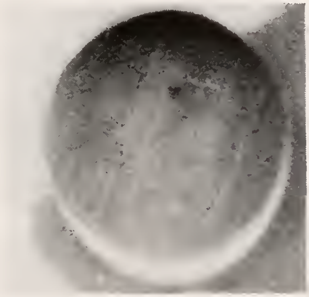


FIGURE 1. The egg of *Rana palustris* 20 minutes after fertilization, showing the small, dark Eispek at the animal pole.

The embryos were fixed in Smith's fluid, sectioned at 10 micra, stained with the "Nuclealfarbung" method of Feulgen, and counterstained with light green (Moore and Ryan, 1940). The sectioned embryos were studied and chromosomal counts made. To check on the interpretations, certain embryos were restained with a triple stain recommended by Dr. S. M. Rose. This consisted of picric acid to stain the yolk and endoderm, blau schwartz to stain cell walls and ectoderm, and Orange G to stain both cytoplasm and mesoderm. The stages used to describe the development of the embryos are those of Pollister and Moore (1937).

In referring to individuals, the specific names will be shortened to *pip* and *pal.* The first name will indicate the egg, and the second name the sperm. If the maternal chromosomes were removed, the first name will be in parentheses, indicating a haploid embryo. For example, pip pal means that a pipiens egg was fertilized by a palustris sperm, resulting in a diploid hybrid. In a (pip) pal embryo the maternal pipiens chromosomes have been removed, resulting in an androgenetic embryo with palustris chromosomes in pipiens cytoplasm.

In a study of androgenetic hybrid embryos, several controls are needed. These controls are the normal diploid embryos, the diploid hybrid embryos and the androgenetic embryos. In order to simplify the description of the experimental material, this material will be divided into two parts. The first part will be a description of those embryos in which the egg of *R. pipiens* was used, and the second part will deal with those embryos in which the egg of *R. palustris* was used.

The Development of (pip) pal Embryos and Their Controls

In this experiment eggs obtained from one Vermont pipiens were used. Some of the eggs were fertilized by sperm from one Vermont pipiens. The remainder of the eggs were fertilized by the combined sperm of two palustris from Woods Hole, Mass. The Eispek was removed from some of the eggs. A total of 204 (pip) pal, 112 (pip) pip, 145 pip pal and 40 pip pip embryos were made and studied. The results from this one large experiment were similar to preliminary experiments when four different pipiens females and five different palustris males were used.

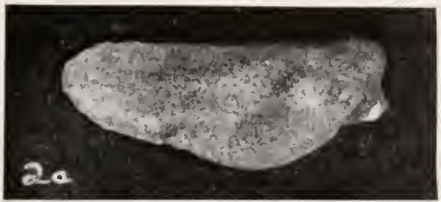
Since the diploid embryos pip pip and the diploid hybrids pip pal were perfectly normal, no description is needed of their development. However, the androgenetic control embryos (pip) pip develop abnormally and a brief description, based on my material, follows. Porter (1939) has described the development of androgenetic pipiens in detail.

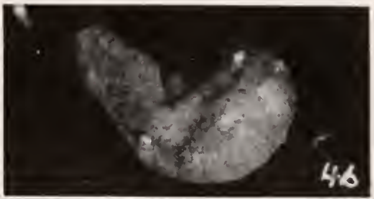
Androgenetic pipiens eggs cleave and gastrulate normally. Their development is delayed during gastrulation. A few embryos begin to cytolyze during the neural fold stage. At the time of closing neural folds many haploids have smaller neural folds than the diploids, and are retarded in development. When the diploid controls pip pip reach stage 19 (heart beat), the haploid (pip) pip are in stage 18 (muscular contraction). When the diploid controls reach stage 20 (gill circulation), the haploids resemble a late stage 18. A few show heart beat and tiny gill projections, but the remainder show neither. All have very large pronephric swellings, wrinkled abdominal epidermis, deep stomadeal pit and a short, broad body (Figs. 2a and 2b). When the diploid controls reach stage 22 (perforated mouths and with the gills almost overgrown by the operculum), the haploids have an open mouth and a small operculum growing over the tiny gill projections. Some of the haploids are very swollen and bloated in appearance, and some are collapsed and wrinkled. When the controls are in stage 23 (horny teeth and with the operculum completely covering the gills), most haploids show no further differentiation than described above. They are either swollen or collapsed and appear to be dying (Figs. 3a and 3b).

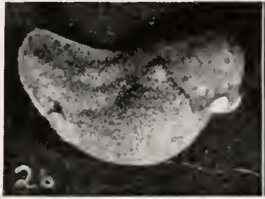
The percentage of haploid survival is very high up to tailbud stage. After this stage many embryos begin to die, and differentiation is retarded and incomplete, even though survival may continue for some time.

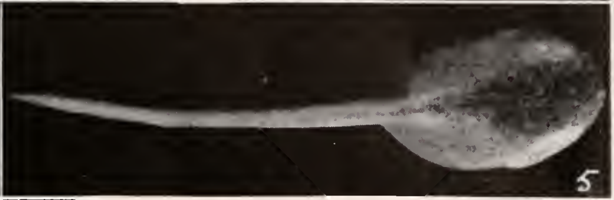
From the foregoing description it is evident that haploid development is distinct from diploid development. Haploids are easily recognized by their characteristic development, which is retarded and abnormal to a varying degree.

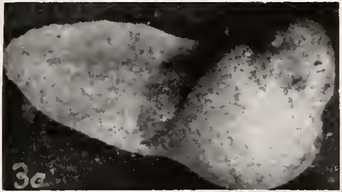
The development of the androgenetic hybrid embryos (pip) pal was markedly different from the control haploid pipiens development. The embryos were retarded during gastrulation so that when the control pip pal embryos had reached an early yolk plug stage, these embryos still had a semicircular dorsal lip. When the (pip) pal embryos reached an early yolk plug stage, some of the yolk cells appeared vacuolated. A small normal yolk plug was formed in all, and up to this time no cytolysis had occurred. By the time the control hybrids were in an early neural fold stage, cytolysis of the haploid hybrids began. The (pip) pal formed a flat

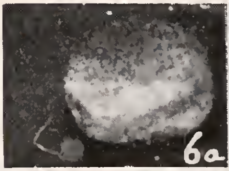


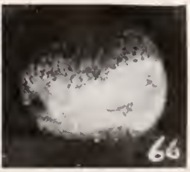


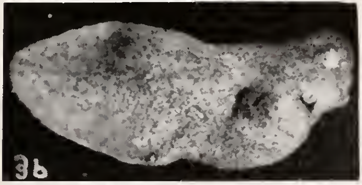


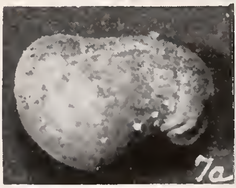


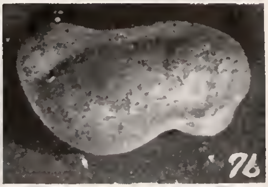


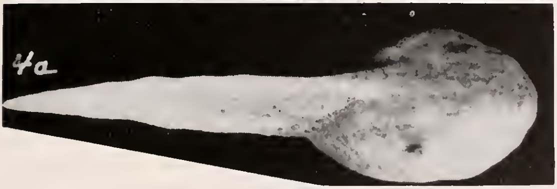


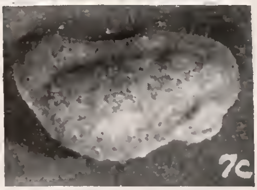












FIGURES 2a and 2b. Androgenetic pipiens embryos whose diploid controls are in stage 20.

FIGURES 3a and 3b. Androgenetic pipiens embryos whose diploid controls are in stage 23.

FIGURES 4a and 4b. Androgenetic pipiens embryos showing the degree of variation in differentiation. Embryos a and b are the same age and from the same experiment.

FIGURE 5. The diploid pipiens control embryo for Figures 4a and 4b.

FIGURE 6a. Elongated (pip) pal neural plate embryo.

FIGURE 6b. A (pip) pal embryo showing the exposed gray cells of the neural plate region.

FIGURE 7. Control embryos for the (pip) pal embryos figured in 6a and 6b. a, (pip) pip. b, pip pip. c, pip pal.

neural plate, and the embryo elongated slightly (Fig. 6a). In those embryos in which the yolk plug was still evident, the yolk plug cells had begun to cytolyze. The wave of cytolysis spread anteriorly, eventually involving the whole neural plate. In embryos in which the yolk plug was a mere slit, a few loose cells appeared on the posterior edge of the neural plate, and again the wave of cytolysis progressed anteriorly. When the vitelline membrane was removed from an embryo, these loose cells came off with it. This revealed the neural plate region as a mass of gray cells, the pigmented ectodermal cells having been sloughed off (Fig. 6b). These (pip) pal embryos can be compared with their controls in Fig. 7.

The (pip) pal embryos were still neural plate embryos when pip pal had closed neural folds. Only 20 (pip) pal survived until the pip pal had reached a tailbud stage. Of these, 3 were still neural plate embryos, 13 showed very slight neural folds and 4 were normal diploid embryos. No further differentiation of (pip) pal embryos occurred, and death followed shortly. This retardation and stoppage of development of (pip) pal embryos is shown in Table I.

TABLE I

A record of the stage of development of (pip)pal embryos and their controls on successive days

Age in days	The stage of development of			
	(pip)pal	(pip)pip	pip pal	pip pip
5th day	11	11–12	12	12
6th day	12	12	12	12–13
7th day	12	12–14	13	13–14
8th day	12–13	13–16	14–15	15–16
9th day	13	14–17	14–16	16–17
10th day	13–14	16–17	16–17	17

Seven (pip) pal embryos were sectioned, varying from a neural plate to a faint neural fold stage, the latter representing the maximum development. A description of these embryos follows after a description of the normal picture in a diploid early neural fold embryo of *R. pipiens*.

A normal embryo is diagrammed in Figure 8. The neural ectoderm is greatly thickened in the anterior and middle regions where it will give rise to brain and neural folds. The notochord in the anterior region is not differentiated. In progressively more posterior sections it gradually becomes differentiated from the mesoderm until it is completely separated from all three layers. The mesoderm in the anterior region continues dorsally as chorda-mesoderm, and extends laterally and somewhat ventrally. Toward the posterior region the mesoderm is completely separated from the notochord and extends a short way laterally. The gut anteriorly has a large lumen which is not roofed by endoderm. Its lumen gradually becomes smaller and completely roofed by endoderm in the posterior region. A liver is present. There are very few cells in mitosis, and no pycnosis nor abnormal nuclei are apparent.

The (pip) pal neural plate embryo is strikingly different. The following description of embryo (pip) pal No. 8, Figure 17, is typical. The neural ectoderm

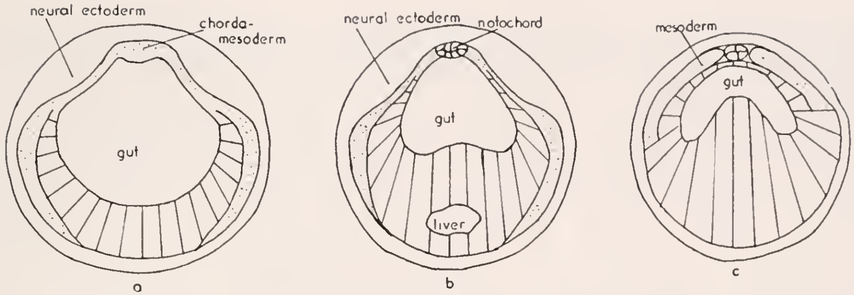


FIGURE 8. Diagram of a diploid pipiens early neural fold embryo, showing an anterior section in a, a middle section in b, and a posterior section in c.

is greatly thickened in the anterior and middle regions of the embryo. Pycnotic nuclei are present in the anterior neural ectoderm which gives rise to brain. In the most anterior region the pycnotic nuclei number about 20 per section. They decrease in number until, in approximately the region where brain and neural tube would join, none are present. In some of these nuclei the chromatin is clumped together. In others the center of the nucleus is faintly stained, and around the periphery is heavily stained chromatin. Still other nuclei are small, condensed and heavily stained.

In the anterior region of the embryo the mesoderm is first distinguishable as a separate layer laterally. It continues dorsally where it fuses with the ectoderm and endoderm. There is no differentiation into chorda-mesoderm. Near the middle of the embryo the mesoderm is separate from the ectoderm and endoderm dorsally and extends laterally and somewhat ventrally. Toward the posterior part of the embryo the mesoderm is a complete layer ventrally as well as dorsally. No notochord is present here or anywhere thoroughout the whole embryo.

The gut along its whole length has a very small lumen which is roofed with endoderm. No liver is present. Both the extent of the mesoderm and the roofing of the gut are characteristic of older diploid closed neural fold embryos, not of neural plate embryos. In contrast to a normal diploid neural plate embryo, many

TABLE II

A comparison of the developmental features of each sectioned (pip)pal embryo with a normal diploid neural plate embryo. The lengths of various organs are measured in micra from the anterior end of each embryo

Embryo	Total length	Gut extends from	Total length of gut	Notochord extends from	Liver extends from
pip pip	1730	130–1510	1390	640–1420	350–650
(pip)pal No. 1	1770	380–1320	950	0	0
(pip)pal No. 2	1890	140–1510	1380	0	0
(pip)pal No. 3	1840	220–1640	1430	0	0
(pip)pal No. 4	1740	180–1470	1300	0	0
(pip)pal No. 6	1700	190–1360	1180	0	0
(pip)pal No. 7	1840	250–1720	1480	0	0
(pip)pal No. 8	1710	80–1500	1430	0	0

cells of the ectoderm and mesoderm were in mitosis. Chromosomal counts showed all 7 embryos to be haploid, with 13 chromosomes. In Table II the developmental features of (pip) pal embryos are summarized and compared with a normal pipiens neural plate embryo.

Embryo (pip) pal No. 6 differed from the other embryos sectioned in that the neural plate cells had cytolyzed. A cross section of this embryo is shown in Figure 18. The dorsal ectoderm is a mass of loose, spongy cells with poorly staining cytoplasm and with condensed, pycnotic nuclei. Many of the ectodermal cells have been sloughed off, as no surface coat is present at the outer surface of the loose cells. The whole length of the neural plate shows cytolyzing cells. From one-quarter to one-third of the way from the anterior end of the embryo, all of the dorsal ectodermal layer and possibly some of the underlying mesoderm is involved in this cytolysis. This is difficult to ascertain, as in this region mesoderm and ectoderm are difficult to separate dorsally. More posteriorly, only the ectoderm is involved. Very few cells of this embryo were in mitosis. In Figure 17 the cytoplasm of the neural ectodermal cells is poorly stained and seems altered in character. This change may well presage the sloughing off of these cells as pictured in Figure 18. Embryo (pip) pal No. 1 also showed cytolysis of the dorsal ectodermal cells, but to a minor extent.

From the foregoing description of (pip) pal embryos it is evident that gastrulation is accomplished, and the three germ layers are formed. Neither notochord nor liver differentiate. The embryos remain in an arrested abnormal neural plate stage. Pycnotic nuclei are present in the neural ectoderm which will give rise to brain. Mitotic activity ceases. The ectodermal cells at the dorsal edge of the blastopore round up and begin to slough off. This cytolysis of the ectodermal cells spreads anteriorly until the whole neural plate is involved, and the embryo then dies.

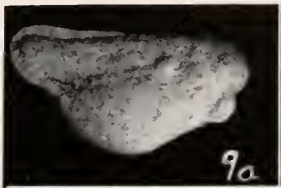
THE DEVELOPMENT OF (PAL) PIP EMBRYOS AND THEIR CONTROLS

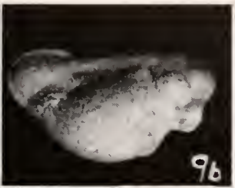
In this cross, 3 experiments were performed at different times. In each experiment eggs were obtained from one palustris female and part of the eggs were fertilized by a palustris male and the remainder by a pipiens male. Thus three different palustris females, 4 palustris males (the combined sperm of two small males were used in one experiment) and 3 pipiens males were used. A total of 304 (pal) pip embryos, 148 (pal) pal, 363 pal pip and 214 pal pal were made and studied.

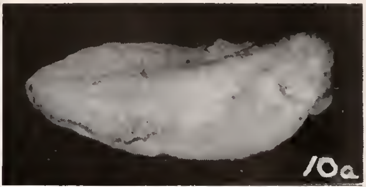
The diploid embryos pal pal and the diploid hybrids pal pip developed perfectly normally. The (pal) pal embryos were similar in their development to (pip) pip embryos. Figures 9 and 10 show haploid palustris tadpoles. They have short, broad tails, large swollen bellies, and microcephalic heads with optic vesicles, olfactory pits and suckers. The (pal) pal embryos differ from (pip) pip in that their survival to later stages (Table IV) and their degree of differentiation is low. Their maximum development is shown in Figure 10. Only 4 of the 148 embryos survived until the control palustris diploids were in stage 21 (cornea transparent). In these (pal) pal the gills developed as a tiny, short filament, the stomadeum was a very small opening, and the heart was observed beating.

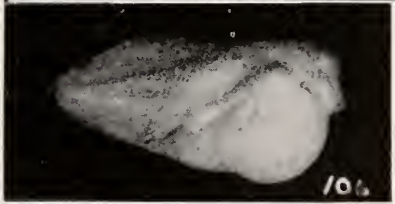
The androgenetic hybrid embryos (pal) pip develop normally up to gastrulation. From the beginning of gastrulation their development is retarded, abnormal and highly variable. The dorsal lip may remain as a crescent and a small neural plate may appear and two small tailbuds may project above the large yolk plug (Fig. 11). These embryos differentiate no further, though they may survive until their controls (pal) pal are in stage 18 and pal pip and pal pal in stage 19. In some embryos a large yolk mass and short anterior projection are present (Fig. 12a).

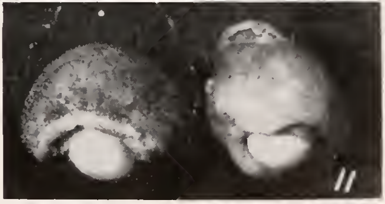
A few embryos formed dorsal and lateral lips of the blastopore, although no ventral lip was present. In these embryos invagination and overgrowth may occur to such an extent that only a tiny yolk plug is left. Low neural folds may form and close (Fig. 12b) and an anterior projection may develop upon which no morphological head structures are discernible (Figs. 12c and 12d). The percentage of

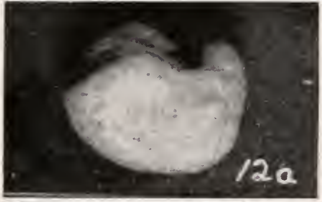


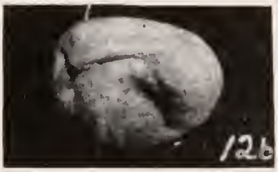


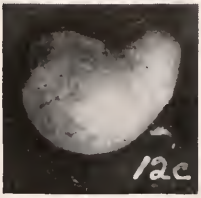


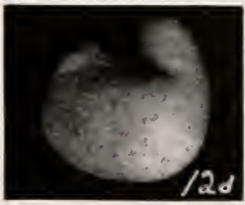


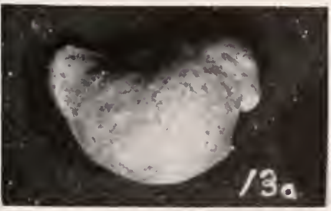


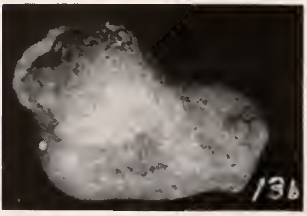












FIGURES 9a, 9b, 10a and 10b. Androgenetic palustris embryos.

FIGURE 11. Two (pal) pip No. 149 embryos showing abnormal gastrulation.

FIGURES 12a and 12b. Two (pal) pip No. 184 embryos, b showing neural folds.

FIGURES 12c and 12d. Two (pal) pip No. 273 embryos, showing anterior projection and short tail.

FIGURES 13a and 13b. Two (pal) pip No. 184 embryos, showing maximum differentiation reached by any (pal) pip embryo.

TABLE III

A record of the percentage of (pal)pip to reach various degrees of differentiation in the different experiments

	Original number	Percentage of (pal)pip showing				
		Incomplete gastrulation	Complete gastrulation	Neural plate	Neural folds	Anterior projection
Exp. 4/24	34	97.1	0.0	—	8.8	0.0
Exp. 4/19	149	98.6	0.0	57.2	18.8	4.5
Exp. 5/2	121	88.2	10.8	66.7	47.7	12.6
Total	304	92.6	4.1	61.4	29.1	7.0

(pal) pip embryos to reach these various degrees of differentiation are shown in Table III.

A few embryos gastrulated completely, but no differentiation of head structures on the anterior projection occurred. Only two (pal) pip out of all 304 embryos showed greater differentiation (Figs. 13a and 13b). Optic vesicles and suckers are recognizable in one. The percentage of survival of (pal) pip embryos is lower than that of (pal) pal and no (pal) pip embryo survived longer than control stage 19 (Table IV). The retardation of development of these androgenetic hybrid embryos is marked and is summarized in Table V.

Fifteen (pal) pip embryos were sectioned. Thirteen of these ranged in development from an early neural plate stage with a large yolk mass to a closed neural

TABLE IV

A comparison of the percentage of survival of (pal)pip embryos and their controls at various stages of development

	Original number	Percentage of survival when the control pal pip were in		
		Stage 13 6th day	Stage 17 9th day	Stage 18–19 12th day
(pal)pip	304	90.6	31.2	11.1
(pal)pal	148	92.1	40.9	20.4
pal pal	214	100.0	99.6	93.6
pal pip	363	98.2	95.0	93.4

TABLE V

A comparison of the stages of development of pal pip and (pal)pip embryos

pal pip	9	10	11	12	14–15	16	17	18–19
(pal)pip	9	10	10	11	11 with neural plate	11 with neural folds	11 with closed neural folds	11 with anterior projection or closed blastopore with projection

fold stage with an anterior projection. Two were the embryos which have been described as the most advanced. The presence or absence of various organs and their lengths are recorded in Table VI, where they are also compared with data from normal pip pip embryos. In addition, each embryo has been graphically reconstructed in Figures 14, 15, and 16. Each embryo is identified by a number. Those with the same initial numbers, for example 250–1 and 250–2, are from the same experiment and fixed at the same time, so that they are of the same age, though of various degrees of differentiation. Some of the sectioned embryos with the initial numbers 149 have been shown as whole embryos in Figure 11, 273 in Figures 12c, 12d and 184 in Figures 12a, b and 13a, b.

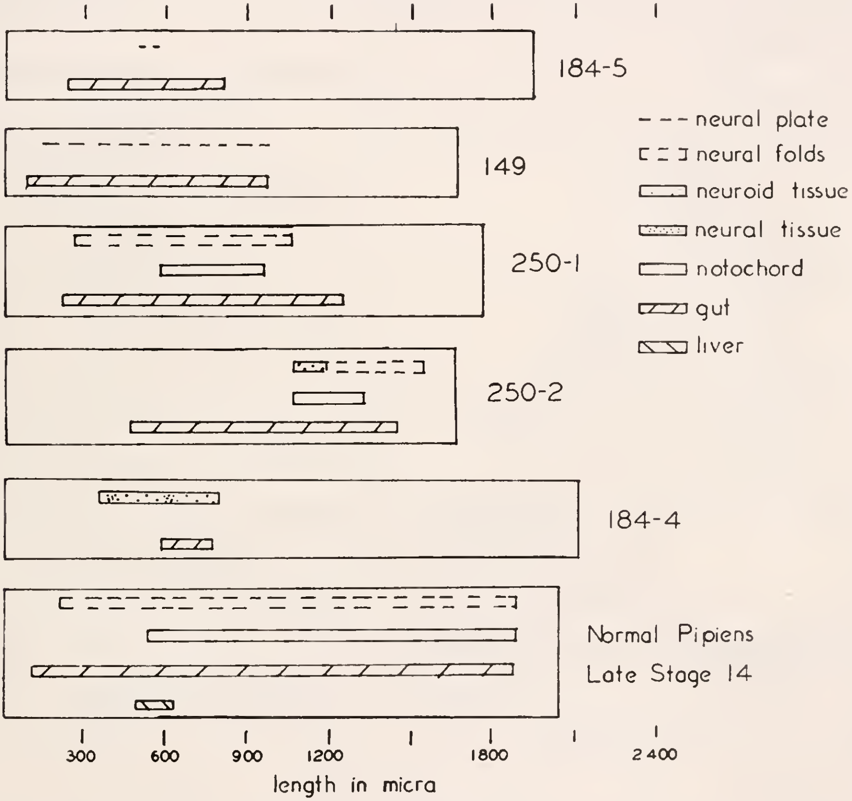


FIGURE 14. Graphic reconstructions of 5. (pal) pip embryos and of a normal late stage 14 pip pip embryo No. 168.

The five least differentiated (pal) pip embryos are graphically reconstructed in Figure 14 and compared with a normal pip pip late stage 14 (neural folds) whose stage of development they most closely resemble. Their control pal pip embryos ranged in age from stage 14 for embryo 149 to stage 16 (closed neural folds) for embryos 250–1 and 250–2, to stage 19 (heart beat) for embryos 184–5 and 184–4.

A large yolk mass protruded dorsally in the posterior half of each embryo except 250–2.

The five (pal) pip embryos which survived until the control pal pip were in either stage 18 (muscular contraction) or stage 19 are graphically reconstructed in Figure 15, and compared with a normal pip pip early stage 16 embryo whose stage of development they most closely resemble. Each embryo had closed neural folds but a large yolk mass protruded dorsally in embryos 184–8C, 184–7B and 238.

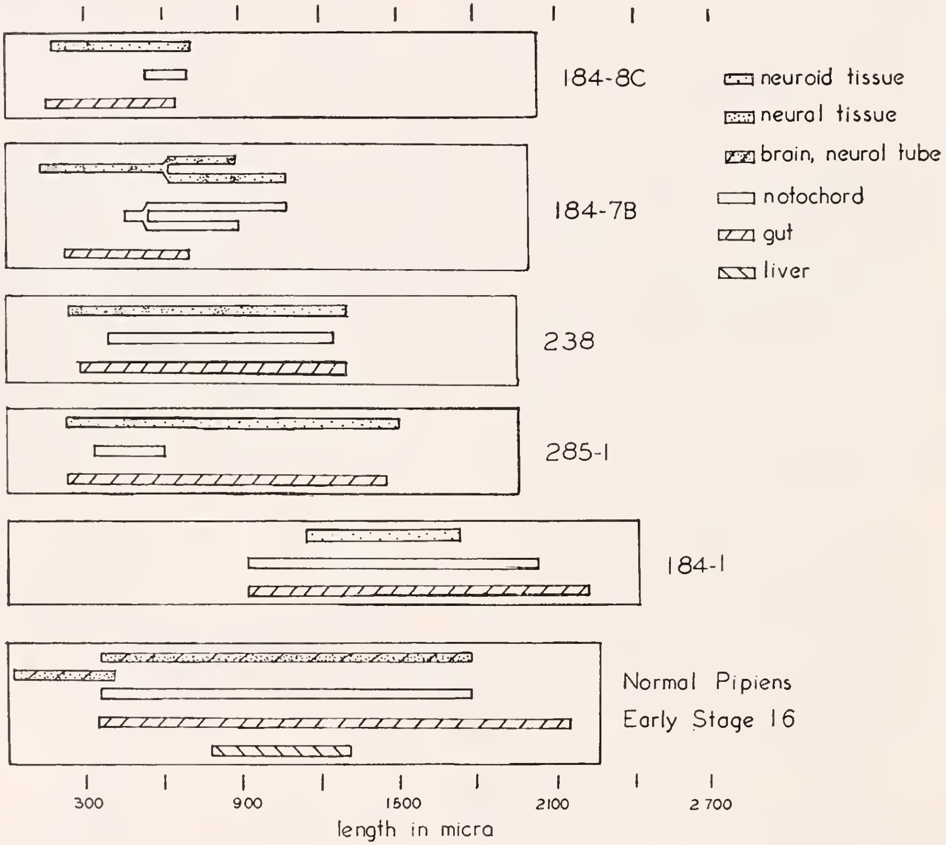


FIGURE 15. Graphic reconstructions of 5 (pal) pip embryos and of a normal early stage 16 pip pip embryo No. 166.

The remaining five (pal) pip embryos also survived until the control pal pip embryos were in either stage 18 or stage 19, but showed greater differentiation. These embryos are graphically reconstructed in Figure 16 and compared with a normal pip pip stage 18 embryo, whose stage of development they most closely resemble. Anterior projections were present in embryos 184–2, 273 and 285–2. Embryos 184–6a and 184–9 were the most advanced (pal) pip embryos shown in Figures 13a and b.

A general description of the internal differentiation of (pal) pip, based on these fifteen sectioned embryos, will now follow.

A neural plate with lateral ectodermal thickenings may differentiate, even though a large yolk plug may be present due to incomplete gastrulation (Fig. 14, embryos 184–5, 149). Neural folds may develop (Fig. 14, embryos 250–1, 250–2) but are very low, with the cells poorly oriented (Fig. 19), contrasting markedly with a normal closing neural fold embryo (Fig. 20). Where these neural folds close, differentiation into neuroid tissue may occur (Fig. 14, embryo 250–2; Fig. 15, embryo 184–1). The term "neuroid tissue" is used to describe a mass of darker staining, poorly differentiated ectodermal cells with scattered pigment throughout. Such neuroid tissue is shown in Figure 21 and can be compared with a normal neural tube in Figure 22.

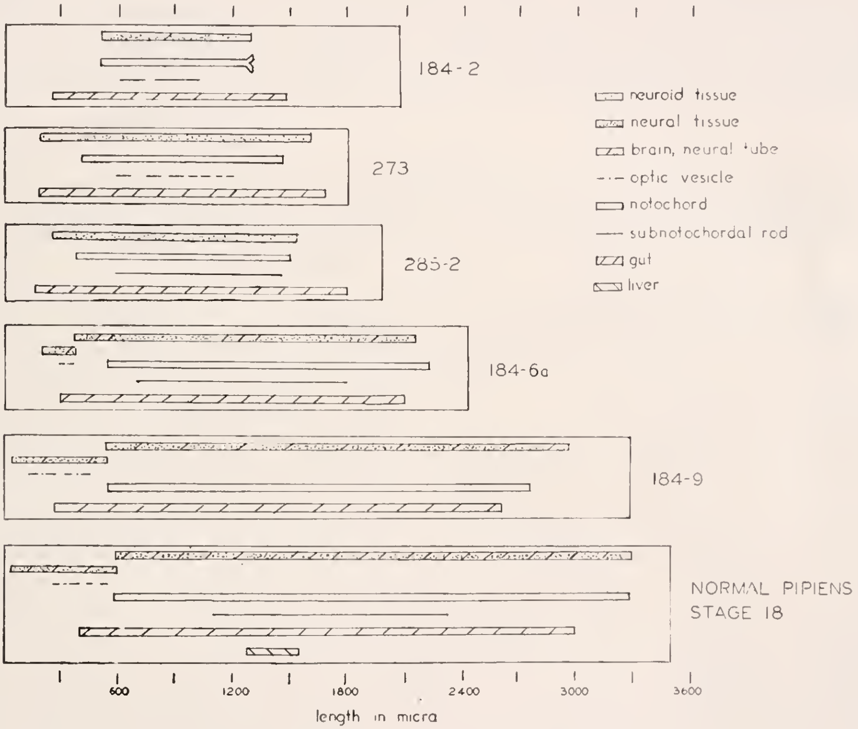

184-2
273
285-2
184-6a
184-9
NORMAL PIPIENS
STAGE 18
neuroid tissue
neural tissue
brain, neural tube
optic vesicle
notochord
subnotochordal rod
gut
liver
600
1200
1800
2400
3000
3600
length in micra

FIGURE 16. Graphic reconstructions of 5 (pal) pip embryos and of a normal stage 18 pip pip embryo No. 613.

In some embryos not only is neuroid tissue present, but also neural tissue. The term "neural tissue" is used to define a group of ectodermal cells which are more or less delimited from the surrounding cells by a membrane, and may or may not show a neurocoel (Fig. 23). It is markedly different from a normal neural tube (Fig. 22). Both neuroid and neural tissue are present in varying amounts in the embryos (Figs. 14–16 and Table VI).

In those embryos in which an anterior projection is formed there is a small amount of neuroid tissue and a large amount of neural tissue (Fig. 16, embryos 184–2, 273 and 285–2). The neural tissue may, at times, closely approximate a neural tube (Fig. 26) and in one such embryo (184–2) a neural tube was present for a short distance. This neural tube is thick and abnormal in appearance (Fig. 27) when it is compared with a normal pipiens neural tube (Fig. 28).

Neural tissue, neural tube, brain and optic vesicles are present in the two most advanced embryos (Fig. 16, embryos 184–6a and 184–9). The brain and optic vesicles are very thick walled and abnormal in appearance (Fig. 29) as can be seen by a comparison with a normal embryo (Fig. 30). One of these embryos (184–9) had a thick walled hind brain and paired otic vesicles (Fig. 31) markedly different from those in a normal embryo (Fig. 32). Its neural tube was very abnormal posteriorly, appearing as a round vesicle with a single layer of cells (Fig. 33).

TABLE VI

A comparison of the lengths of organs found in each sectioned (pal)pip embryo with those of normal pip pip embryos. Length is in micra. c = control pal pip. st = stage of development

(pal)pip embryo number	Total length	Length of								
		Neural plate	Neural folds	Brain	Neuroid and neural tissue	Neural tube	Notochord	Subnotochordal rod	Gut	Liver
184–5 c-st 19	1960	80					0		570	0
149 c-st 14	1670	830					0		890	0
250–1 c-st 16	1770		790				400		1010	0
250–2 c-st 16	1670				480		240		980	0
184–4 c-st 19	2120				400		0		170	0
184–8c c-st 19	2050				530		170		500	0
184–7b c-st 19	2010				760 470 940		440		480	0
238 c-st 19	1970				1070		860		1030	0
285–1 c-18	1970				1290		270		1240	0

TABLE VI.—*Continued*

(pal)pip embryo number	Total length	Length of								
		Neural plate	Neural folds	Brain	Neuroid and neural tissue	Neural tube	Notochord	Subnotochordal rod	Gut	Liver
184–1 c-st 19	2440				580		1130		1310	0
184–2 c-st 19	2080				780		800 770 800	350	1230	0
273 c-st 18	1810				1430		1070	510	1490	0
285–2 c-st 18	1970				1290		1120	880	1640	0
184–6a c-st 19	2440			170	1780		1690	1120	1820	0
184–9 c-st 19	3290			500		2430	2230	0	2350	0
pip pip 168 st 14	2050		1700				1380		1680	140
pip pip 166 st 16	2250			410		1510	1510		1870	290
pip pip 613 st 18	3540			570		2730	2730	1240	2610	270

A notochord was absent in the (pal) pip neural plate embryos and in one closed neural fold embryo (Fig. 14, embryo 184–4). The notochord was present in the remaining closed neural fold embryos but it was highly variable in length and bore no relationship to the length of the neuroid and neural tissue (Figs. 14 and 15). In the most highly defferentiated (pal) pip embryos (Fig. 16) a well developed notochord and subnotochordal rod were present (Fig. 26).

A gut with a small lumen was present in all embryos. No liver was present in any (pal) pip embryos. A mesodermal layer was absent in the most poorly differentiated embryo 184–5, but present in all the rest of the embryos of Figs. 14 and 15 and embryo 285–2 of Fig. 16. The remaining embryos of Fig. 16 showed somites. A short pronephric duct was present in the most advanced embryo (184–9). A heart was not present in any (pal) pip embryo.

Chromosomal counts showed the following embryos to be haploid: 184–5, 250–2 and 184–4 of Figure 14; 184–7B and 285–1 of Figure 15; 273 and 184–6a of Figure 16. The remaining 8 (pal) pip embryos were either mitotically inactive or with nuclei so faintly stained that chromosomal counts could not be made. They are believed to be haploid as their differentiation was similar to other (pal) pip embryos in which chromosomal counts could be made.

Pycnotic nuclei and loose cells were noted in some of the least differentiated and in some of the most highly differentiated embryos. Figure 24 shows such loose ectodermal and mesodermal cells which were scattered in a large fluid filled space about the notochord of embryo 184–1, Figure 15. The pycnotic nuclei (Fig. 25) of these loose cells show either peripheral clumping of the chromatin or they are small, condensed and darkly staining. Pycnotic nuclei were present in ectodermal, mesodermal and endodermal cells (embryo 184–6a, Fig. 16).

Oedema occurred in some of the less differentiated as well as in some of the most highly differentiated embryos. The oedematous spaces were within the ectoderm, between the ectoderm and endoderm, or between the ectoderm and mesoderm. One such large space is shown in Figure 33.

From the foregoing description it is evident that even though gastrulation in (pal) pip embryos may be incomplete, a neural plate with lateral ectodermal thickenings is formed. A shallow neural groove and low neural folds with poorly oriented ectodermal cells is then formed. When the folds close, neuroid and neural tissue are formed. With more complete gastrulation, differentiation into a recognizable neural tube and even into brain may occur. A notochord develops. A gut but no liver is present. A mesodermal layer and even somites are present. Oedema may occur. Pycnosis may be associated with the breakdown of tissues.

Discussion

The discussion will be devoted to the consideration of haploidy in amphibians and of the results obtained by combining a haploid set of chromosomes of one species with the cytoplasm of a different species.

The abnormal development of haploid parthenogenetic and androgenetic amphibian embryos has been ascribed to the condition of haploidy *per se* or to the presence of unmasked lethal or semi-lethal genes. It would seem logical that both factors play a role. A characteristic haploid syndrome may be present. Haploid tadpoles are usually small, with short bodies, broad tails, abnormal eyes, a poorly looped gut and oedema. The degree of differentiation is, however, highly variable. In a large group of (pip) pip embryos the degree of abnormality may vary from extreme to slight. Two such embryos are shown in Figures 4a and

---

Plate I

Figure 17. Section of embryo (pip) pal No. 8, 610 micra from anterior end, showing thickened neural ectoderm.

Figure 18. Section of embryo (pip) pal No. 6, 920 micra from anterior end, showing cytolyzing neural plate cells.

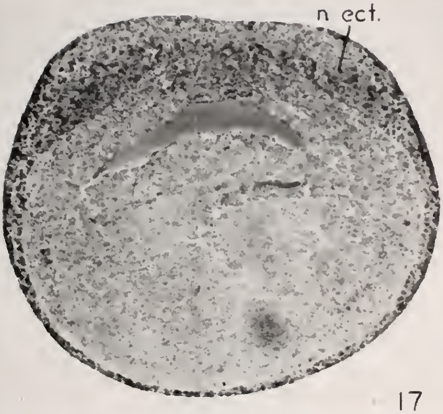
Figure 19. Section of embryo (pal) pip 250–1, 750 micra from anterior end, showing neural folds.

Figure 20. Section of embryo pip pip 168, 530 micra from anterior end showing normal neural folds.

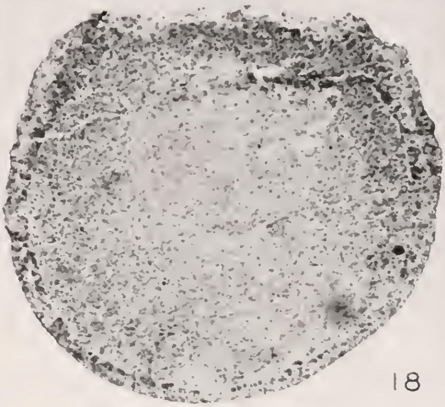
Figure 21. Section of embryo (pal) pip 250–2, 1100 micra from anterior end, showing neuroid tissue.

Figure 22. Section of embryo pip pip 166, 830 micra from anterior end, showing normal neural tube.

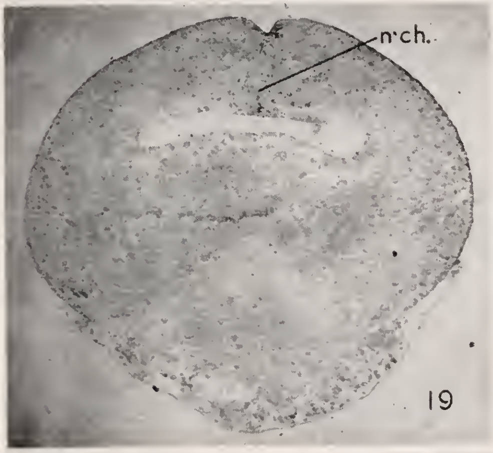
PLATE 1



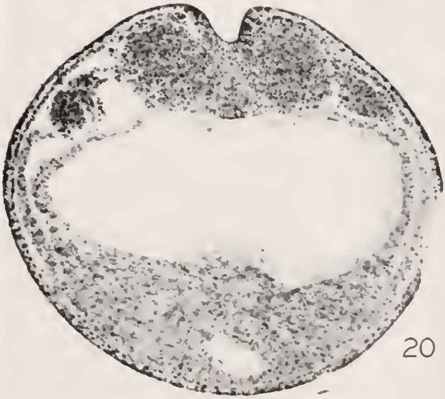
17



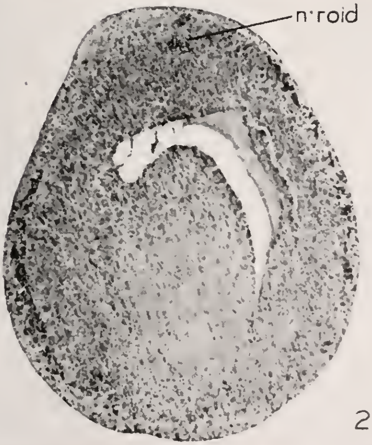
18



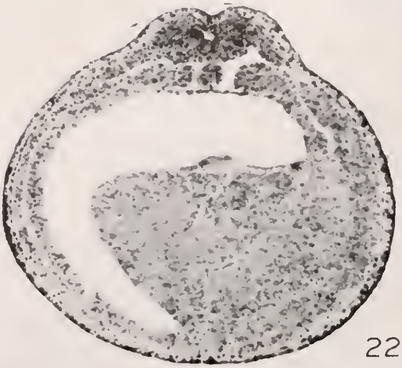
19



20



21



22

b, which can be compared with their normal control in Figure 5. Such variations among haploid Triturus larvae have been described by Baltzer (1922), Baltzer and de Roche (1936) and Fankhauser (1930). These *variations* of development, superimposed on the haploid syndrome, are probably due to the different genetic constitution of each embryo. In the haploid condition recessive genes are unmasked. If any of these are embryonic lethals or semi-lethals, their effects will be observed in development. In haploid embryos in which the majority of the genes are of the normal type, it is possible that differentiation may go far and that even metamorphosis may be reached. This view is supported by the fact that Baltzer (1922) and Fankhauser (1938) described a metamorphosed haploid *T. taeniatus.* With increasing numbers of semi-lethal and lethal genes, haploid differentiation would become poorer and death occur at earlier stages. Thus genetic differences would explain the variability of haploid development.

As evidence against this hypothesis the viability of diploid parthenogenetic frogs (Parmenter, 1933) has been cited. If these diploids were produced by a doubling of the chromosomes at the first mitotic cleavage, completely homozygous individuals would result. Such individuals, homozygous for semi-lethals and lethals, should be inviable. As a matter of fact, only half of the few parthenogenetic diploids of Parmenter metamorphosed, the remainder dying at an early stage. Kawamura (1939) found that in a large number of diploid parthenogenetic frogs only 17 per cent metamorphosed, and even these showed slight irregularities. The remaining diploids were abnormal and his drawings of some of these diploids markedly resemble embryos with the haploid syndrome. Such results tend to support the hypothesis that unmasked recessive genes play a role in abnormal haploid development. Those embryos which have unmasked lethals and semi-lethals can not survive in either the haploid or diploid condition.

In those parthenogenetic cases where diploidy is restored by the retention of two sets of maternal chromosomes through the suppression of a meiotic division, a certain degree of heterozygosity would result and such diploids might have normal viability.

The injurious effect of haploidy varies from species to species in amphibia. In my experiments, haploid pipiens embryos live longer and differentiate further than haploid palustris embryos. Androgenetic *T. taeniatus* have reached metamorphosis (Baltzer, 1922), whereas androgenetic *T. palmatus* and *alpestris* survive only until limb bud stages (Fankhauser, 1930; Baltzer and de Roche, 1936). Haploid *R. esculenta* (Dalcq, 1932) usually form neurulae, and some develop tiny

---

PLATE II

FIGURE 23. Section of embryo (pal) pip 285–1, 430 micra from anterior end, showing neural tissue.

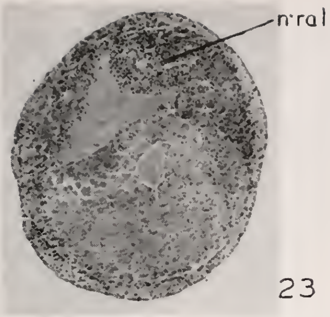
FIGURE 24. Section of embryo (pal) pip 184–1, 1600 micra from anterior end, showing loose cells in fluid filled space about notochord.

FIGURE 25. Pycnotic nuclei present in the loose cells shown in Figure 24.

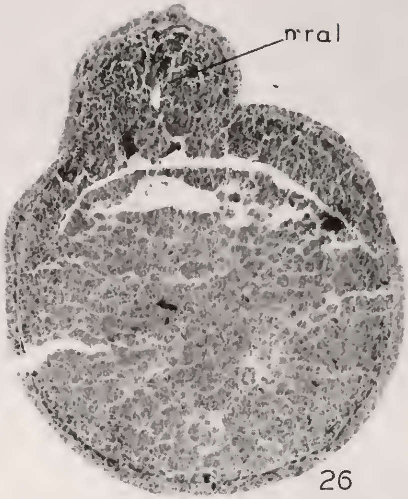
FIGURE 26. Section of embryo (pal) pip 184–2, 960 micra from anterior end, showing neural tissue, notochord, and subnotochordal rod.

FIGURE 27. Section of embryo (pal) pip 184–2, 890 micra from anterior end, showing neural tube.

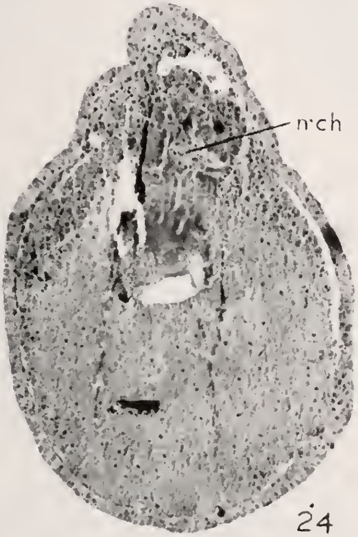
FIGURE 28. Section of embryo pip pip 613, 1270 micra from anterior end, showing normal neural tube.



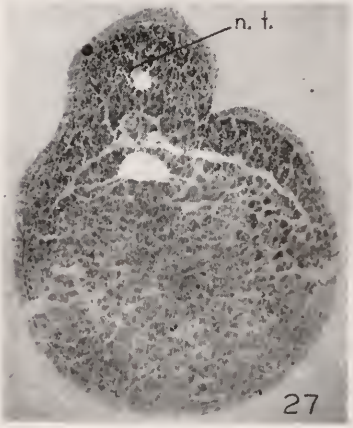
23



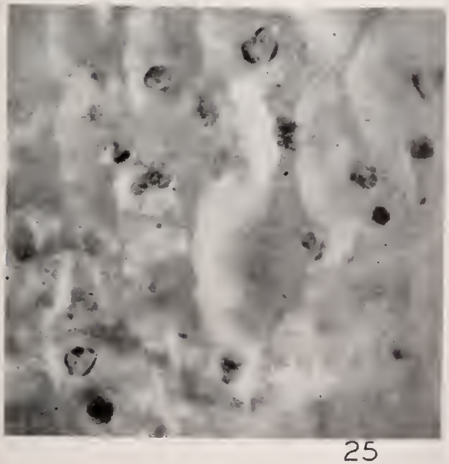
26



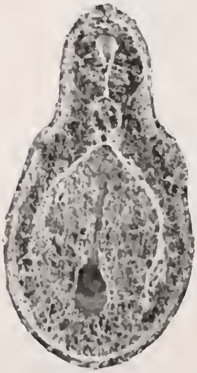
24



27



25



28

gills. Fankhauser (1937) and Kaylor (1940) found that in haploid *T. pyrroghaster* approximately half of the embryos die as gastrulae but that others go on to a fore-limb bud stage. Stauffer (1945) found that androgenetic axolotl survive only as long as the gastrula and neurula stage. Griffiths (1941) showed that the majority of androgenetic *T. viridescens* embryos die as blastulae and gastrulae, but about 10 per cent form neurulae.

Death during late blastula and early gastrula stages is probably not due to haploidy but to abnormal chromosomal distributions and mitotic aberrations. Because of polyspermy in Triturus, a balanced set of chromosomes may not be present and Fankhauser (1934) has pointed out that such a balanced set appears to be needed for gastrulation and later development. Development to a late blastula or early gastrula stage appears to be under maternal cytoplasmic control, and further differentiation is under genic control. Boveri (1907) noted this in dispermic sea urchin eggs and Moore (1941) has discussed this concept in relation to frog hybrids. All of these findings corroborate the probability that the abnormal development of haploid embryos is partially a result of genic action.

From the data presented it is evident that the results obtained by combining a haploid set of palustris chromosomes with the cytoplasm of *R. pipiens* are markedly different from those results when the palustris chromosomes are in their own cytoplasm. These (pip) pal embryos die at an earlier stage than (pal) pal or (pip) pip. Some process or processes in differentiation are either lacking or are unable to carry out their normal functions. For example, the absence of the differentiation of the neural tube may be explained embryologically by several possibilities. (1). The organizer may be in a weakened condition, so that a neural tube can not be induced. (2). The organizer may be normal but the neural ectoderm may be weakly competent and thus no neural tube is formed. (3). The organizer may be weak *and* the neural ectoderm poorly competent, therefore no neural tube is formed. A definite answer to these three possibilities can only be obtained by xenoplastic implants and transplants of the materials involved.

Similarly, the results obtained by combining a haploid set of pipiens chromosomes with the cytoplasm of *R. palustris* are markedly different from those results when the pipiens chromosomes are in their own cytoplasm. These (pal) pip embryos differentiate much more abnormally than (pip) pip or (pal) pal. The neural ectoderm is particularly affected, as a recognizable brain and neural tube are rarely formed. In these embryos, as in (pip) pal, the competence of the neural ectoderm may be lowered or the inductive capacity of the organizer may be weakened,

---

PLATE III

FIGURE 29. Section of embryo (pal) pip 184–9, 210 micra from anterior end, showing brain and optic vesicles.

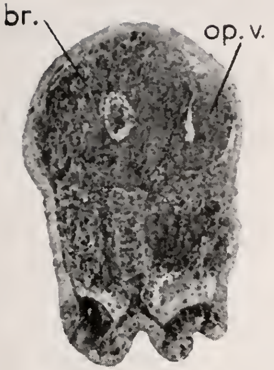
FIGURE 30. Section of embryo pip pip 613, 420 micra from anterior end, showing normal brain and optic vesicles.

FIGURE 31. Section of embryo (pal) pip 184–9, 740 micra from anterior end, showing otic vesicle.

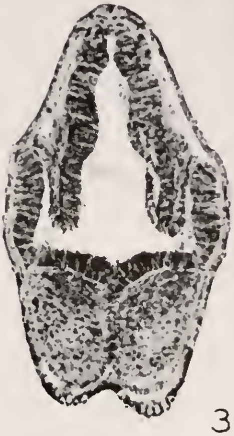
FIGURE 32. Section of embryo pip pip 613, 800 micra from anterior end, showing normal otic vesicles.

FIGURE 33. Section of embryo (pal) pip 184–9, 2350 micra from anterior end, showing abnormal neural tube and large oedematous space.

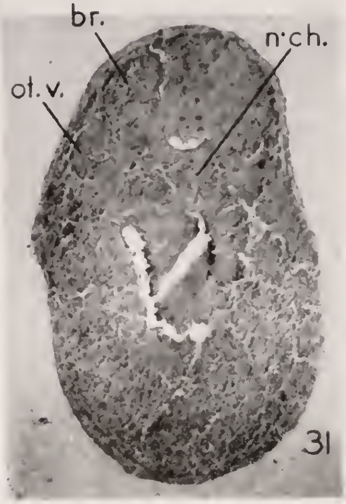
PLATE III



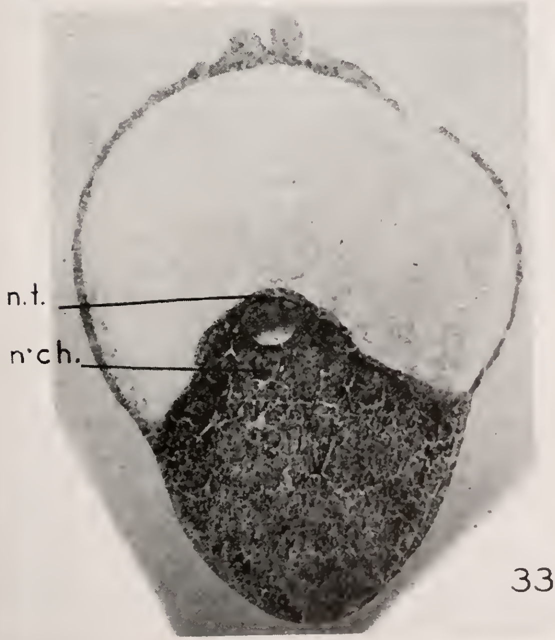
29



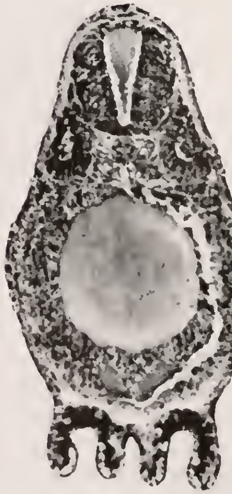
30



31



33



32

ABBREVIATIONS FOR PLATES

br., brain
op. v., optic vesicle
ot. v., otic vesicle
n'ch., notochord
n. ect., neural ectoderm
n'ral, neural tissue
n'roid, neuroid tissue
n.t., neural tube

resulting in abnormal development and early death. Both lowered competence and weakened organizer together may play a role. In the (pal) pip neurulae a regional difference in the strength of the organizer may be present, in that the head organizer may be very weak or even absent in most of the embryos. Differentiation of the anterior end of the gut is probably abnormal, since a liver diverticulum never develops. Implantation of the organizer and transplantation of the presumptive neural ectoderm of (pal) pip embryos to a foreign host would clarify the roles they play in the abnormal development of these embryos.

From previous work on amphibian androgenetic hybrids it has long been known that when a haploid set of chromosomes from one species is combined with the cytoplasm of another species, the haploid development differs from that when the chromosomes and cytoplasm are of the same species. For example, Baltzer (1920) has shown that androgenetic *T. taeniatus* embryos form larvae and even reach metamorphosis, but androgenetic hybrids between *T. taeniatus* ♀ X *T. palmatus* ♂ develop only as far as larvae with forelimbs. It is interesting to note that diploid hybrids between these two species are capable of metamorphosing.

From the data presented it is evident that the androgenetic hybrids are alike in showing pycnosis. In (pip) pal embryos the pycnotic nuclei are confined to the neural ectoderm, a region which is mitotically very active in these embryos. In (pal) pip embryos the pycnosis may ultimately involve all tissues but the notochord. The presence of pycnosis has often been described in androgenetic embryos of other amphibia, in androgenetic hybrids and in diploid hybrids. Stauffer (1945) found pycnosis in the blastula and gastrula cells of androgenetic white or black axololts. In the few embryos which formed neurulae he found pycnotic nuclei in the neural tube, brain and mesoderm. Curry (1936) found pycnotic nuclei in the head mesenchyme of the neurulae of androgenetic hybrids between *T. alpestris* females and *T. cristatus* males. He also described the brain lumen as being filled with degenerated cell material. The neural tube was without a lumen and it and the brain were poorly developed. This description of internal development is similar to that of (pal) pip development. Baltzer (1938) described pycnosis in the gastrulae of diploid hybrids between *T. cristatus* females and *T. palmatus* males. Only a few of these hybrids develop as far as neurulae.

From the foregoing it is apparent that pycnosis may be characteristic of tissues which are approaching death and have ceased differentiation and begun to disintegrate. It is a manifestation of abnormal processes which are going on in the embryos. Leuchtenberger (1950) has shown that definite chemical changes are occurring in the nucleus during pycnosis. These changes involve a progessive loss of protein with a depolymerization and progressive loss of the desoxyribose nucleic acid. Thus, during pycnosis, significant chemical changes are occurring in the nuclear material.

From the data presented it is evident that not only do the androgenetic hybrids (pip) pal and (pal) pip differ from their androgenetic controls (pip) pip and (pal) pal, but that they also differ from each other. The haploid set of paternal chromosomes of either pipiens or palustris, when present in its own specific cytoplasm, is capable of normal gastrulation, neurulation and the formation of tadpoles. If pipiens cytoplasm is substituted for palustris cytoplasm, the haploid set of palustris chromosomes is only capable of carrying differentiation to the neural plate stage.

Since the genes in (pip) pal embryos are similar to those in (pal) pal embryos, the difference in the development between (pip) pal and (pal) pal is probably due to differences between pipiens and palustris cytoplasm. In (pal) pal embryos the palustris cytoplasm is able to supply normal materials to the palustris genes for their synthesis. In addition the palustris genes have the palustris cytoplasm to act upon and utilize in differentiation. In the pipiens cytoplasm certain substrates may be the same as those in the palustris cytoplasm, even as certain of their genes are homologous, since the reciprocal diploid hybrids develop normally. (Homologous spotting genes have been shown to be present in pipiens and palustris by Moore, 1943.) Other substrates in the pipiens cytoplasm may be species specific. Baltzer (1930) suggested from his studies of androgenetic hybrids that species specific substances existed in the cytoplasm. The palustris genes may not be able to utilize these species specific substrates, so that development ceases; or they may be able to utilize these substrates in forming compounds which may then act as anti-metabolites (Woolley, 1947), resulting in abnormal development. Similarly, in the reverse cross (pal) pip, the pipiens genes may not be able to utilize certain of the palustris substrates, or these substrates may act as anti-metabolites when utilized, with resulting abnormal development.

Since (pip) pal and (pal) pip embryos differ from each other in their development, different morphogenetic processes are probably upset. The uniformity of (pip) pal development suggests that a certain morphogenetic substance (or substances), which may contribute to one early major developmental step, is lacking or abnormal. For example, such a substance might be involved in the conversion of chordamesoderm to notochord. In this case one might think that the palustris gene controlling this major developmental step is not able to work with the pipiens substrate to form the necessary materials. In the reverse cross, (pal) pip, the great variation in development may indicate that several different substances which contribute to different developmental steps may be lacking or abnormal. In this case one might say that several pipiens genes may be involved in a variety of developmental steps, but are unable to work with the palustris substrates, resulting in abnormal and variable development, since a larger number of different genes and substrates are involved.

Differences between reciprocal androgenetic hybrids and their androgenetic controls have not been previously described and analyzed in detail. Baltzer (1933) has recorded differences between reciprocal androgenetic hybrids of *T. palmatus* and *T. alpestris*. Dalton (1946) noted differences between the reciprocal androgenetic hybrids and androgenetic controls among *T. torosus*, *T. granulosis* and *T. rivularis* embryos but this work dealt primarily with the role cytoplasmic properties may play in transplanted androgenetic hybrid tissue. Dalton found that such transplanted tissue shows the influence of the cytoplasm in the number and dispersion of melanophores. Hadorn (1936) had earlier attempted to show cytoplasmic influence in transplanted androgenetic hybrid epidermis, but with equivocal results.

The results obtained in this study suggest that the abnormal development and death of haploid amphibian embryos is due to both haploidy *per se* and to unmasked lethal and semi-lethal genes. In the development of androgenetic hybrids not only may these two factors be operative but, in addition, the cytoplasmic substrates may play a role.

SUMMARY

The development of androgenetic *R. pipiens* and *R. palustris* embryos has been briefly described. A detailed study of their androgenetic hybrids has been made.

The (pip) pal embryos develop uniformly to an early neural fold stage, then differentiation ceases, the neural plate cells are sloughed off and the embryos die.

The (pal) pip embryos develop uniformly until early gastrulae. Thereafter their development is highly variable. Gastrulation is usually incomplete, and a short neural plate may be formed. In a few embryos closure of the neural folds may occur and neuroid and neural tissue be formed. An occasional embryo is capable of slightly further differentiation, with an abnormal brain and neural tube, and optic and otic vesicles.

The effects of haploidy and the results obtained by combining a haploid set of chromosomes of one species with the cytoplasm of a different species are discussed.

LITERATURE CITED

BALTZER, F., 1920. Über die experimentelle Erzeugung und die Entwicklung von Triton-Bastarden ohne mütterliches Kernmaterial. *Verh. Schweiz. Natf. Ges.*, **101**: 217–220.

BALTZER, F., 1922. Über die Herstellung und Aufzucht eines haploiden Triton taeniatus. *Verh. Schweiz. Natf. Ges.*, **103**: 248–249.

BALTZER, F., 1930. Die Zussamenarbeit von Plasma und Kern in der tierischen Entwicklung. *Mitt. Naturforsch. Ges.* Bern.

BALTZER, F., 1933. Über die Entwicklung von Triton-Bastarden ohne Eikern. *Verh. Dtsch. Zool. Ges.*, **35**: 119–126.

BALTZER, F., 1938. Die Frühenentwicklung der Bastards *Triton cristatus* ♀ × *Triton palmatus* ♂. *Rev. Suisse Zool.*, **45**: 391–394.

BALTZER, F. AND V. DE ROCHE, 1936. Über die Entwicklungsfähigkeit haploider *Triton alpestris*-keime und über die Aufhebung der Entwicklungshemmung bei Geweben letaler bastardmerogonischer Kombination durch Transplantation in einer normalen Wirt. *Rev. Suisse Zool.*, **43**: 495–505.

BOVERI, T., 1907. Zellenstudien. VI. Die Entwicklung dispermer Seeigeleier. Ein Beitrag zur Befruchtungslehre und zur Theorie des Kernes. *Jen. Z. Naturwiss.*, **43**: 1–292.

CONKLIN, E. G., 1905. Mosaic development in Ascidian eggs. *Jour. Exp. Zool.*, **2**: 145–223.

CONKLIN, E. G., 1908. Mechanism of heredity. *Science*, **27**: 89–99.

CURRY, H. A., 1936. Über die Entkernung des Triton Eies durch Absaugen des Eifleckes und die Entwicklung des Tritonmerogons *Triton alpestris* (♀) × *Triton cristatus* ♂. *Roux Arch.*, **134**: 694–715.

DALCQ, A., 1932. Contribution à l'analyse des fonctions nucléaires dans l'ontogénèse de la Grenouille. IV. Modifications de la Formule chromosomiale. *Arch. de Biol.*, **43**: 343–366.

DALTON, H. C., 1946. The role of nucleus and cytoplasm in development of pigment patterns in Triturus. *Jour. Exp. Zool.*, **103**: 169–200.

FANKHAUSER, G., 1930. Entwicklungspotenzen diploidkerniger halften des ungefurchten Tritoneies. *Roux Arch.*, **122**: 671–735.

FANKHAUSER, G., 1934. Cytological studies on egg fragments of the salamander Triton. V. Chromosome number and chromosome individuality in the cleavage mitoses of merogonic fragments. *Jour. Exp. Zool.*, **68**: 1–58.

FANKHAUSER, G., 1937. The production and development of haploid salamander larvae. *Jour. Hered.*, **28**: 2–15.

FANKHAUSER, G. 1938. The microscopical anatomy of metamorphosis in a haploid salamander, *Triton taeniatus* Laur., *Jour. Morph.*, **62**: 393–413.

GRIFFITHS, R. B., 1941. Triploidy (and haploidy) in the newt, *Tr. viridescens*, induced by refrigeration of fertilized eggs. *Genetics*, **26**: 69–88.

HADORN, E., 1936. Übertragung von Artmerkmalen durch das entkernte Eiplasma beim merogonischen Triton-Bastard, palmatus-Plasma × cristatus-Kern. *Verh. Dtsch. Zool. Ges.*, **38**: 97–104.

HERTWIG, P., 1916. Durch Radiumbestrahlung verursachte Entwicklung von halbkernigen Triton-und Fischembryonen. *Arch. mikro. Anat.*, **87**, II: 63–122.

KAWAMURA, T., 1939. Artificial parthenogenesis in the frog. I. Chromosome numbers and their relation to cleavage histories. *Jour. Sci. Hirosima Univ.*, Ser B., Div. 1 (*Zool*), **6** (7/9): 115–218.

KAYLOR, C. T., 1940. Studies on experimental haploidy in salamander larvae. I. Experiments with eggs of the newt, *Triturus pyrrhogaster*. *Biol. Bull.*, **79**: 397–408.

LEUCHTENBERGER, C., 1950. A cytochemical study of pycnotic nuclear degeneration. *Chromosoma*, **3**: 449–475.

MOORE, B. C. AND E. W. RYAN, 1940. Chromosomes of frog eggs and embryos stained by the Feulgen method to avoid excessive staining of yolk granules. *Anat. Rec.*, **78** Suppl. 1: 122.

MOORE, J. A., 1941. Developmental rate of hybrid frogs. *Jour. Exp. Zool.*, **86**: 405–422.

MOORE, J. A., 1943. Corresponding genes in spotted frogs. *Jour. Heredity*, **34**: 3–7.

PARMENTER, C. L., 1933. Haploid, diploid, triploid and tetraploid chromosome numbers, and their origin in parthenogentically developed larvae and frogs of *Rana pipiens* and *R. palustris*. *Jour. Exp. Zool.*, **66**: 409–453.

POLLISTER, A. W. AND J. A. MOORE, 1937. Tables for the normal development of *Rana sylvatica*. *Anat. Rec.*, **68**: 489–496.

PORTER, K. R., 1939. Androgenetic development of the egg of *Rana pipiens*. *Biol. Bull.*, **77**: 233–257.

STAUFFER, E., 1945. Versuche zur experimentellen Herstellung haploider Axolotl-Merogone. *Rev. Suisse Zool.*, **52**: 231–327.

WILSON, E. B., 1904. Experimental studies on germinal localization. I. The germ regions in the egg of Dentalium. *Jour. Exp. Zool.*, **1**: 1–72.

WILSON, E. B., 1904. Experimental studies on germinal localization. II. Experiments on the cleavage—mosaic in Patella and Dentalium. *Jour. Exp. Zool.*, **1**: 197–268.

WOOLLEY, D. W., 1947. Recent advances in the study of biological competition between structurally related compounds. *Physiol Rev.*, **27**: 308–333.

ACTION OF ESTRONE AND PROGESTERONE ON NUCLEAR VOLUME (STUDIED BY APPLYING THE KARYOMETRIC-STATISTICAL METHOD)

CARLOS ALBERTO SALVATORE

Clinica Ginecólogica, Faculdade de Medicina, Universidade de São Paulo, Brazil [1]

The action of hormones in tissues is demonstrated by the appearance of specific changes (hypertrophy, hyperplasia, edema, etc.) which can, under certain circumstances, be induced by different hormones.

Variations of nuclear volumes, exactly pointed out by the Karyometric-statistical method as mentioned in preceding papers (Salvatore, 1948a, 1948b), may be, in themselves, a suggestion of the action of certain hormones. Having this in mind, we decided to begin a series of researches in order to observe the action of sexual hormones on nuclear volumes, to find the maximal and minimal doses able to bring about duplication of nuclear volume in uterine cells and possibly also, to find the doses of hormone able to induce a nuclear polyploidy.

As already stated in preceding papers (Salvatore and Schreiber, 1947a; Salvatore, 1948a, 1948b), nuclei of muscular cells in female pregnant rats attain volumes 4, 6 and 8 times the size of the smallest volume found; whereas, endometrial cells have their nuclei generally remaining double in volume.

Recent Karyometric-statistical researches point to the close relationship between the nuclear volume and the number of chromosomes (genome); hence, the present study possibly may be able to show the action of chemically known substances (hormones) on the genic materials influencing their reproduction.

The Karyometric-statistical method also constitutes an excellent means of elucidating the nature of nuclear hypertrophy; for some authors claim that nuclear hypertrophy is due to trophic phenomena, while others attribute it to phenomena of a genic or chromosomic nature.

In our present report, we shall study the action of estrone and progesterone on the nuclear volume of endrometrial glands of the uterus of castrated female rats by applying the Karyometric-statistical method.

MATERIAL AND TECHNIQUE

We used in our present experiment, 10 full grown albino female rats (*B. Wistar*) which were submitted to ovariectomy. After one month of castration, we injected in two rats, intramuscularly, 0.5 cc. of an oily solution of estrone (Oestrone Abbott) containing 100 I.U. estrone, for 3 days, totalling 300 I.U. (record 3 and 4); in two female rats, 250 I.U. were injected per day for 4 days, totalling 1000 I.U. (records 5 and 6); two female rats with an oily solution of progesterone (Lutocyclin Ciba) in a daily dose of 0.5 mgr. for 2 days, total dose equaling 1.0 mgr. (records

---

[1] Director of Gynecological Department: Professor José Medina.

TABLE I

N° of protocol	Stages	Dose	Modal value		Nuclear size																			N° of nuclei	Mitotic index
					8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5		
12	Castrate	—	523		4	10	17	*47*	35	15	10	7	5											150	—
13	Castrate	—	539		1	4	10	*43*	39	29	20	4												150	—
3	Estrone treatment	300 I.U.		1150							1	2	23	*51*	42	22	7	1	1					150	1.1%
4	Estrone treatment	300 I.U.		1177								11	15	*56*	36	25	6	1						150	0.5%
5	Estrone treatment	1000 I.U.		1150								5	24	*55*	33	16	6	2	*4*	*4*	—	—	1	150	1.5%
6	Estrone treatment	1000 I.U.		1150							1	6	21	*61*	40	15	4	2						150	1.3%
7	Progesterone treat.	1 mgr.	572		2	7	22	46	53	13	7													150	—
9	Progesterone treat.	1 mgr.	523			7	27	*58*	30	20	11	6	1											150	—
10	Progesterone treat.	2 mgr.	508			6	43	*59*	28	11	8	5												150	—
11	Progesterone treat.	2 mgr.	523			7	26	*50*	29	21	8	6	3											150	—

TABLE II

Stages	Average of modal value		Average in μ^3		Nuclear size																			N° of nuclei
					8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5	
Castrate	531.0		$88.1\mu^3$		5	14	27	*90*	74	44	30	11	5											300
Estrone		1156.7		$192.0\mu^3$							2	24	83	*223*	151	78	23	6	5	4			1	600
Progesterone	531.5		$88.2\mu^3$		7	27	138	*213*	140	65	34	17	4											600

7 and 9); and two female rats getting 0.5 mgr. daily for 4 days, total dose equaling 2 mgr. (records 10 and 11). Two female rats (records 12 and 13) acted as controls.

After the last injection, slides of vaginal secretion, obtained from all animals, showed diestrum in castrated controls and in those injected with progesterone, and typical estrus in those injected with estrone.

The animals were killed 6 hours after the last injection. Fragments of uterine material were fixed on alcoholic Bouin and mounted on paraffin and sections measuring 10 micra were stained with hematoxylin-eosin.

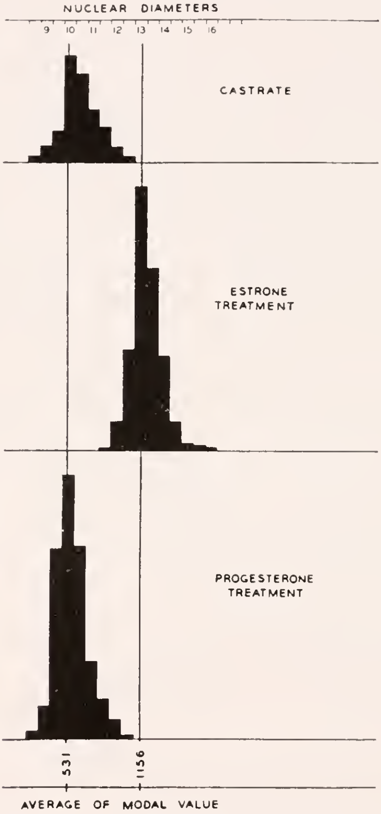


FIGURE 1. Histograms representing the frequency of nuclear volumes.

The Karyometric-statistical method was applied according to the same principle used in previous publications (Salvatore and Schreiber, 1947a, 1947b; Salvatore, 1948a, 1948b).

With a magnification of 1800 diameters, outlines were drawn by camera lucida, 150 nuclei for each sample. Arithmetical means of the diameters of nuclei were grouped into frequency classes; nuclear volumes and modal values were calculated by applying the same formulas used in preceding works (Salvatore, 1948b) and are represented in Table I, together with the mitotic indexes (number of mitoses per 100 nuclei measured).

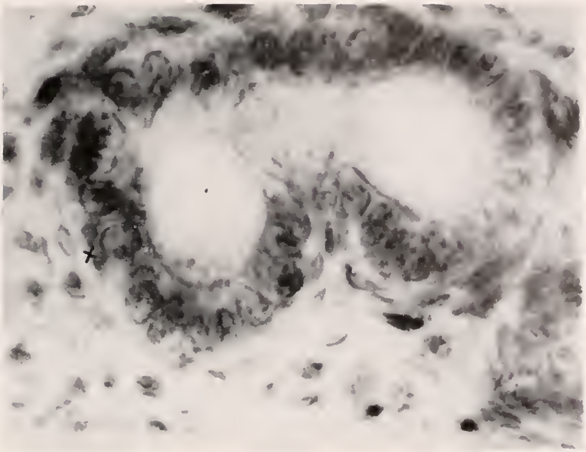


FIGURE 2. Nuclei of cells of the glands pertaining to uterus of castrated animals (control) (Prot. 12) (× 1100).

With the help of results from addition of the frequencies of the arithmetical means of nuclear diameters pertaining to samples of the castrated group, the castrated group injected with estrone, and the castrated group with progesterone (Table II), average histograms were designed, as represented in Figure 1.

Knowing that on an amplification of 1800 times, 1 mm. is equal to 0.55 micron, the nuclear volume of cubic micra was calculated and registered in Table II.

RESULTS

Analyzing Table II, one notices that in the controls as well as in the animals injected with progesterone, arithmetical means of the drawings of nuclei of endo-

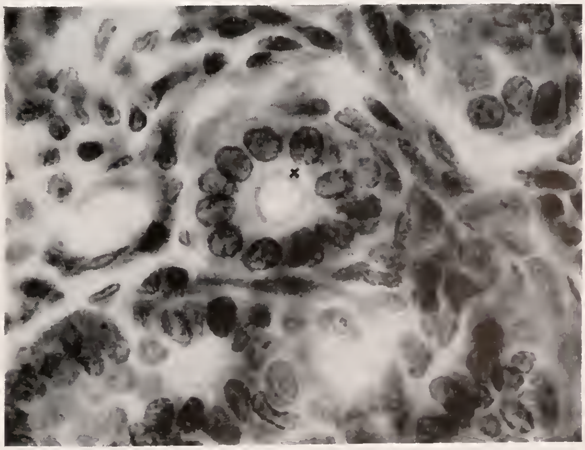


FIGURE 3. Nuclei of cells of the glands pertaining to uterus during progesterone treatment (Prot. 9) (× 1100). The size of these nuclei is the same as that of the control.

metrial glandular cells present only one maximum of frequency, represented by the modal means value of 531.0 and 531.5 or 88.1 and 88.2 cubic micra, respectively.

As noticed in Figure 1, the histogram of controls as well as of those injected with progesterone presents only a unimodal curve, thus demonstrating the homogeneity of nuclear sizes (Figs. 2 and 3).

The same is to be said for nuclear volumes of animals injected with estrone, the histogram of which is also unimodal (Figs. 1 and 4), but of a modal mean value of 1156.7 or 192.0 cubic micra. Only in record 5 (Table I) is found a general delineation of another mode.

The relationship among modes of the control groups and of those injected with estrone stands as 1 :2. No intermediate modal values (1.5), in a 1 :2 progression, were found as in preceding researches.

With both doses of estrone, it was observed that all nuclei had the same reaction, namely, doubling their initial volumes.

Progesterone, in the doses employed, had no action on nuclear volumes, which remained identical with those of the control group.

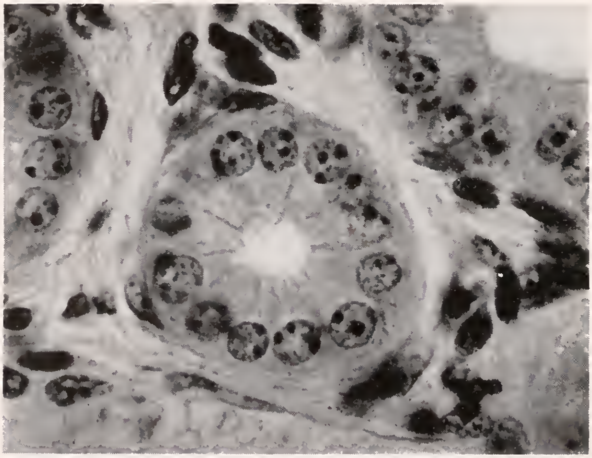


FIGURE 4. Nuclei of cells of the glands of the uterus during oestrone treatment (Prot. 5) (× 1100).

COMMENTS

The action of estrone and progesterone on uterine tissues is perfectly established by now, through works of Corner and Allen (1929), Hisaw and Leonard (1930), Clauberg (1930), Bureau (1939) and Sammartino and Nogues (1945), which allows us to state that oestrogens are responsible for phenomena of uterine growth while progesterone is responsible for changes of structures previously acted on by oestrogens.

Orville and Overholser (1938) reported a typical hypertrophy of muscular cells brought about through estrone injections on castrated female rats.

Pfeiffer and Hooker (1944) obtained nuclear hypertrophy of endometrium estrone cells in female ovariectomized monkeys, by using oestradiol benzoate, or oestradiol benzoate and progesterone simultaneously, and also with the use of progesterone alone.

The action of progesterone on castrated animals not previously submitted to an oestrogen treatment, has already been pointed out by several authors, among them Hisaw (1935), Hisaw, Greep and Fevold (1937), Selye (1940) and Hooker (1940).

More recently, Forbes and Hooker (1947) verified that progesterone alone changes fusiform nuclei of the stroma into vesicular ones.

The results of the present report not only support the conclusion that oestrone brings about nuclear hypertrophy in endometrial cells, but also confirms the results of our researches (Salvatore and Schreiber, 1947a; Salvatore, 1948b) that hypertrophy may be perfectly evaluated by applying the Karyometric-statistical method.

The photographs of nuclei pertaining to uterine endometrial cells of animals injected with oestrone, 1100 times amplified, show a large difference of nuclear sizes (Figs. 2, 3 and 4) when compared to those of the control group. Thus it can be considered as a reliable *test* for oestrone. We have also noticed that the doses of progesterone used by us in our present experiment have no action on the nuclear volume.

The Karyometric-statistical method also established the fact that nuclear hypertrophy is essentially of a chromosomal nature and not consequent to imbibition phenomena, for the modal values are in the progression of 1:2.

Thus, all nuclei which, in the castrated animal, remain resting (basic volume, probably diploid) undergo an interphasic growth, ending by the duplication of their initial volumes.

The Karyometric researches began with Jacobj (1926) showing that physiologically the nuclei of different tissues grow "rhythmically" under the action of normal growth stimuli.

Later the rhythmic growth of nuclear volume, following a geometrical progression 1:2:4:8, was confirmed by Hertwig (1938–39), Wermel (1933), D'Ancona (1941), Sauser, Muller, Keller and Geitler (quoted by Schreiber, 1946) and Hintzsche (1945, 1946, 1949).

Dawson (1948), studying the renal tubules of the "Australian desert frog," not only noticed the existence of uninucleated (diploid unity), binucleated, trinucleated and quadrinucleated cells, but other cells with progressive nuclear sizes as well.

Besides, Jacobj (1926) noticed that during spermatogenesis, a rhythmical reduction in nuclear volumes occurs (4:2:1). The same phenomenon was noticed in the liver during development of carp (*Cyprinus carpus* var. *specularis*) by B. Schreiber and Angeletti (1940) and in liver of tadpoles belonging to *Bufo vulgaris* by G. Schreiber and M. R. Schreiber (1941).

All of these researches as well as, more recently, those of Biesela, Poyner and Painter (1942) on neoplastic tissues, and those of Schreiber (1946, 1947) on polyploid plant of coffee, as well as in ophidian spermatogenesis, clearly show the genic nature of rhythmical growth, *i.e.*, "an intimate correlation between nuclear volume and the chromosomic number."

The results of preceding researches on the nuclear growth on the endometrial and muscular cells belonging to uterine of female rats, during the oestral and gravidic cycle (Salvatore, 1948a, 1948b), during the puerperal involution (Salvatore, 1949), and in ovaric tissues (Salvatore and Schreiber, 1947b) not only confirm the results obtained by several authors but show as well that nuclear hypertrophy is of a chromosomic nature and not due to simple trophic phenomena.

The present report points out that the Karyometric-statistical method can be applied in endocrinology not only as a "test" (for it demonstrated very precisely the action of oestrogens—Oestrone—on the nuclear volumes), but also because as already stated, it clears up the real nature of nuclear hypertrophy.

Once established through cyto-genetic researches that "Duplication of nuclear volume expresses the reproduction of genes," we may insist that "Oestrone acts on the genes," inducing their reproduction.

Conclusions

1. The Karyometric-statistical method can be applied in endocrinology as a test for the determination of the action of oestrogens on uterine tissues.

2. Oestrone induces interphasic growths on nuclei pertaining to endometrial glandular cells of castrated female rats. This growth brings about a duplication of volume (1:2).

According to cyto-genetic researches which show an intimate dependence of the nuclear volumes and number of chromosomes, it is possible that "Oestrone acts on the genes, determining their reproduction." On the other hand, this fact emphasizes the notion that nuclear hypertrophy is essentially of chromosomal nature.

3. Progesterone has no action at all on the nuclear volume of endometrial cells, which remains identical to that of the controls.

LITERATURE CITED

Biesela, J., H. Poyner and T. Painter, 1942. Nuclear phenomena in mouse cancers. *Univ. Texas Publ.*, **42–43**: 1–68.

Bureau, V., 1939. L'action de l'oestrone et de la progesterone dur la corne uterine de la lapine etudiée par la methode á la colchicine. *Compt. Rend. Soc. Biol.*, **130**: 933–934.

Clauberg, C., 1930. Das Hormon des corpus luteum. *Zbl. Gynäk.*, **54**: 2757–2765.

Corner, G. and W. Allen, 1929. Physiology of the corpus luteum. *Am. J. Phys.*, **88**: 326–360.

D'Ancona, U., 1941. Sul poliploidismo della cellula epatiche. *Boll. Soc. Ital. Biol. Sper.*, **16**: 49–50.

Dawson, A. B., 1948. Variations in the number and size of nuclei in the cells of the kidney tubules of an Australian desert frog. *Anat. Record*, **102**: 393–402.

Forbes, T. R. and C. W. Hooker, 1947. A method for the bio-assay of minute amounts of progestin. *Anat. Record*, **97**: 335–336.

Hertwig, G., 1938–39. Abweichungen von dem Verdoppelungswachstum der Zellkerne und ihre Deutung. *Anat. Anz.*, **87**: 65–73.

Hintzsche, E., 1945. Die Kerngrösse der Follikelepithel und der Granulosaluteinzellen im menschlichen Eierstock. *Monat. Gebur. u. Gynek.*, **120**: 200–221.

Hintzsche, E., 1946. Polyploide und Amitose in Geweben von Säugetieren. *Arch. Julius Klaus-Stift.*, **21**: 299–303.

Hintzsche, E., 1949. Zyklische Änderungen der Kerngrösse in Oberflächenepithel und Drüsen des menschlichen Uterus. *Gynaecologia*, **128**: 270–285.

Hisaw, F., 1935. The physiology of menstruation in macacus Rhesus monkeys. *Am. Journ. Obst. and Gynec.*, **29**: 638–659.

Hisaw, F., R. O. Greep and H. L. Fevold, 1937. Effects of progesterone on the female genital tract after castration attrophy. *Proc. Soc. Exp. Biol. and Med.*, **36**: 840–855.

Hisaw, F. and S. Leonard, 1930. Relation of the follicular and corpus luteum hormones in the production of progestational proliferation of the rabbit uterus. *Am. J. Phys.*, **92**: 574–584.

Hooker, C. W., 1940. Effects of progesterone upon the uterus of the mouse. *Proc. Soc. Exp. Biol. and Med.*, **45**: 270–272.

Jacobj, W., 1926. Über das Wachstum der Zellen nach einem Gesetz der konstanten Proportionen. *Münch. Med. Woch.*, **20**: 859–865.

Orville, B. L. and M. O. Overholser, 1938. Hyperplasia and hypertrophy of the uterine musculature in ovariectomized rats following estrone injections. *Anat. Record*, **70**: 401–411.

Pfeiffer, C. A. and C. W. Hooker, 1944. Responses of Stromal nuclei of the macaque endometrium to estrogen and progesterone. *Yale J. Biol. and Med.*, **17**: 35–43.

Salvatore, C. A., 1948a. A cytological examination of uterine growth during the estrous cycle and artifically induced estrus. *Rev. Bras. Biol.*, **8**: 505–523.

Salvatore, C. A., 1948b. A cytological examination of uterine growth during pregnancy. *Endocrinology*, **43**: 355–370.

Salvatore, C. A., 1949. A cytological examination of puerperal uterine involution. *Rev. Bras. Biol.*, **9**: 187–199.

Salvatore, C. A. and G. Schreiber, 1947a. Pesquisas cariometricas no ciclo estral e gravidico. *Mem. Inst. Butantan.*, **20**: 39–78.

Salvatore, C. A. and G. Schreiber, 1947b. Estudo cariometrico das celulas foliculares e luteinicas. *Mem. Inst. Butantan.*, **20**: 355–375.

Sammartino, R. and A. E. Nogues, 1945. Modo de accion del estradiol sobre el utero de conejas, cobayas y ratas castradas. *Obst. y Ginec. Latino Americana*, **3**: 489–509.

Schreiber, B. and S. Angeletti, 1940. Rhythmic increase and decrease of nuclear volume of the hepatic cell of the carp, *Cyprinus carpus* var specularis. *Anat. Record*, **76**: 431–439.

Schreiber, G., 1946. Estudo cariometrico dos poliploides de Coffea. *Bragantia.*, **7**: 279–298.

Schreiber, G., 1947. O crescimento interfasico do nucleo. *Mem. Inst. Butantan.*, **20**: 113–180.

Schreiber, G. and M. R. Schreiber, 1941. Diminuição ritmica do volume nuclear do figado do pancreas nos girinos de anuros. *Bol. Fac. Filos. C. L. Univ. S. Paulo.*, **21**: **5**: 234–240.

Selye, H., 1940. Activity of progesterone in spayed females not pretreated with estrin. *Proc. Soc. Exp. Biol. and Med.*, **43**: 343–344.

Wermel, E., 1933. Studien über Zellengrösse und Zellenwachstum. *Zeitschr. Zellf. und mikr. Anat.*, **17**: 505–513.

NUTRITIONAL REQUIREMENTS OF THE EARLY CHICK EMBRYO. III. THE METABOLIC BASIS OF MORPHOGENESIS AND DIFFERENTIATION AS REVEALED BY THE USE OF INHIBITORS[1]

NELSON T. SPRATT, JR.

The University of Minnesota[2]

INTRODUCTION

In an attempt to learn something about the metabolic changes underlying the developmental processes of morphogenesis and histogenesis, a study of the nutritional requirements of the embryo was begun several years ago (Spratt, 1947). It was thought that a better understanding of the control mechanisms which characterize the differentiation process could be obtained by an examination and comparison of the nutritional properties of differentiating cells with "undifferentiated" and already differentiated cells. Subsequent studies have shown that both initiation and maintenance of particular cellular movements and differentiations of the early chick embryo are dependent upon an adequate nutrient environment, and that different developmental activities have both quantitatively and qualitatively different environmental requirements (Spratt, 1948a, 1948b, 1949a, and 1950). Furthermore, it was shown that differences in metabolism of the completed organs and tissues of the adult are already present during their development.

A further analysis of the biochemical reactions underlying the various developmental activities has been attempted by the use of metabolic inhibitors, substances which have been shown to block or interfere with enzyme activity. Differences in the effects of inhibitors on different embryonic processes would, presumably, indicate differences in the metabolic pathways being used.

Correlation of the effects of inhibitors with those produced by several other environmental changes on particular developmental processes may enable us to construct a better over-all picture of the metabolic basis of embryonic development, even though many important details are lacking. Evidence to be presented, coupled with that provided by preceeding studies, enables us to make the following tentative generalization.

Regions of the chick embryo where cells are undergoing active and rapid differentiation (*e.g.*, the node, the fore-brain, etc.) are more dependent upon an adequate nutrient environment than are regions already differentiated or "undifferentiated." In other words, the pattern of metabolic (enzymatic) activity seems to be directly related to the pattern of differentiation activity.

---

[1] Aided by a grant from the National Institutes of Health, U. S. Public Health Service.

[2] I am grateful for the excellent facilities provided by The Johns Hopkins University where this investigation was done.

EXPERIMENTAL METHODS

Approximately 600 chick blastoderms (definitive primitive streak to 8-somite stages) were removed from incubated eggs and explanted to the surface of synthetic media *in vitro*. Operative techniques, culture methods and methods of preparing media were the same as those previously described (Spratt, 1947, 1948a and 1949a). Compositions of the various control and experimental media will be given below. Results have been recorded in the form of camera lucida drawings of the living explants, made by the author. Most of the explants were dissected under saline; a few were fixed, stained and sectioned for study of histological details.

PROCEDURE AND RESULTS

In studying the effects of inhibitors on early development, two types of control series were set up for each experiment: (1) embryos explanted to non-nutrient, Ringer media and (2) embryos explanted to glucose-Ringer media. An additional type of control series was set up for each experiment on the effects of monoiodoacetate and sodium fluoride: (3) embryos explanted to sodium pyruvate-Ringer media.

A. Development on Control Media

(1) Non-nutrient, Ringer medium. The medium has the following composition, expressed in grams per 100 ml. of medium: NaCl 0.9, KCl 0.042, $CaCl_2$ 0.024, $Na_2HPO_4 \cdot 12H_2O$ 0.0145, KH_2PO_4 0.0026, $NaHCO_3$ 0.055, Agar 0.35.

Explants to the non-nutrient medium begin to degenerate after an initial lag period (2–4 hrs.). The characteristic feature of the degenerative process is a breakdown of structural organization at the organ and tissue levels and dispersal of the cells (Spratt, 1947, 1948a and 1949a). The spatial pattern and time course of the degenerative process is illustrated in Table I. The region most sensitive to extreme starvation is the node, the center of most active morphogenesis and histogenesis during the first two days' development. A second region of high susceptibility is the anterior end of the embryonic axis, the head-fold, where fore-gut, fore-brain and optic vesicle formation occur.

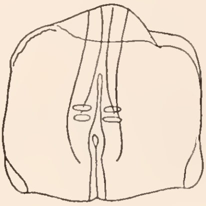
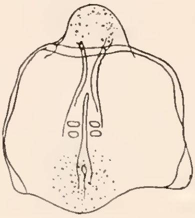
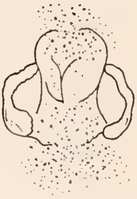
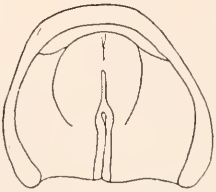
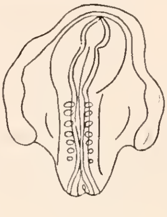
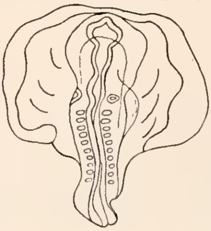
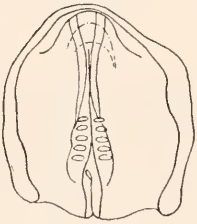
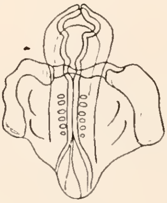
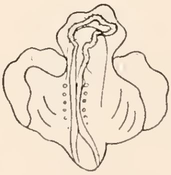
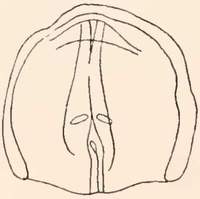
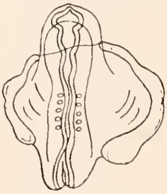
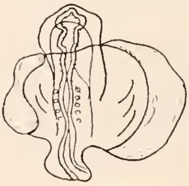
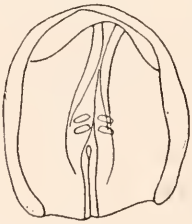
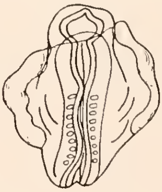
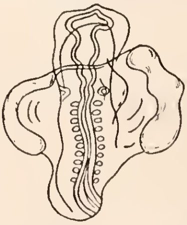
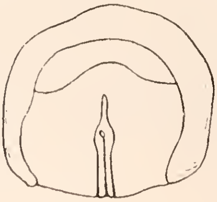
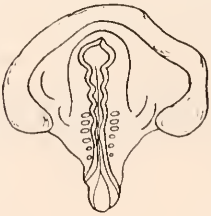
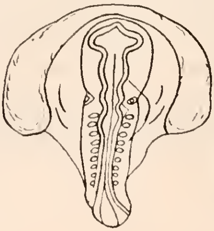
(2) Glucose-Ringer medium. This is simply the non-nutrient medium containing 10^{-3} to $5 \times 10^{-2} M$ d-glucose. In most instances, $10^{-2} M$ glucose was used.

Explants to the glucose medium undergo morphogenesis and differentiation as illustrated by the typical example in Table I: (a) regression of the primitive streak, formation and elongation of the notochord, (b) formation of the head-fold and neural plate, (c) development of the brain, spinal cord, optic vesicles, otocysts and somites, (d) formation and pulsation of the heart, etc. In the absence of an exogenous nitrogen source, the extent of histogenesis is inferior to that obtained in embryos explanted to yolk-albumen media (Spratt, 1947).

The effects of partial carbohydrate starvation (accomplished by decreasing the glucose concentration or by using a less efficiently utilized sugar) were again observed. The results confirm those previously reported (Spratt, 1949a and 1950) in showing that the embryonic region most sensitive to the nutritionally poor environment is the node, with the head-fold region the next most sensitive.

TABLE I

Control series

MEDIUM	NO. EXPL	STAGE EXPLANTED	GENERALIZED RESULTS	
			$10^{\pm}$ HOURS IN VITRO	$20^{\pm}$ HOURS IN VITRO
RINGER-BUFFER	56			
RINGER-BUFFER GLUCOSE 10^{-3}–5×10^{-2}M	140			
RINGER-BUFFER PYRUVATE 10^{-2}–4×10^{-2}M	105			
RINGER-BUFFER LACTATE 2×10^{-2}M	32			
RINGER-BUFFER PYRUVATE 2×10^{-2}M + GLUCOSE 10^{-3}M	28			
RINGER-BUFFER PYRUVATE 2×10^{-2}M + GLUCOSE 2.5×10^{-4}M	4			

(3) Pyruvate-Ringer medium. The non-nutrient medium containing 10^{-2} to $4 \times 10^{-2} M$ sodium pyruvate. In most instances, $2 \times 10^{-2} M$ was the concentration used.

The type of development which occurs on the pyruvate control medium is illustrated in Table I. Although pyruvate can be utilized for supporting some of the early developmental changes, it is inferior to glucose (Spratt, 1949b and 1950). After the first 15–20 hours' cultivation practically all further development ceases and the embryos shrink to an appreciably smaller size than the corresponding glucose controls. Histogenesis of the brain and heart is also inferior. Development on sodium lactate media ($2 \times 10^{-2} M$) is approximately equivalent to that on pyruvate. Some observations indicate that lactate is slightly superior (Table I) but this needs further study.

B. Development on Media Containing Combinations of Glucose and Pyruvate or Lactate

Seven experiments in which a sub-minimal amount of glucose ($2.5 \times 10^{-4} M$) was added to either a minimal ($5 \times 10^{-3} M$) or optimal ($2 \times 10^{-2} M$) pyruvate medium were done.[3]

Results of two experiments are shown in Table I. Here both minmial ($10^{-3} M$) and sub-minimal ($2.5 \times 10^{-4} M$) amounts of glucose plus $2 \times 10^{-2} M$ pyruvate result in development indistinguishable from that of the glucose (10^{-3} to $10^{-2} M$) controls. In general, when both glucose and pyruvate (even at molar concentration ratios of 1:20 or 1:80, respectively) are present in the medium, the embryos are similar in their extent of development to those utilizing glucose alone—not dwarfed like the pyruvate controls. Combinations of sodium lactate and glucose have yielded similar results.

C. Metabolic Inhibitor Effects

(1) Monoiodoacetate (sodium salt). In these and all other inhibitor experiments, a measured amount of a sterilized stock solution of the inhibitor was added to the medium after it had been autoclaved and cooled to about 40° C. The pH of the medium was adjusted to 7.5–8.5 with NaOH (8.0 in most experiments). The pH was measured with the Beckman pH meter at the beginning and end of each experiment and found to remain constant in almost all instances.

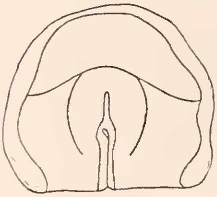
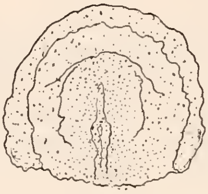
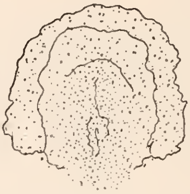
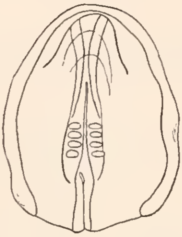
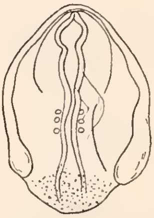
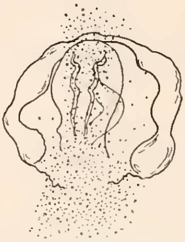
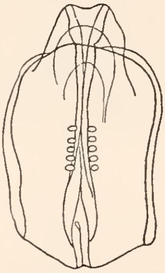
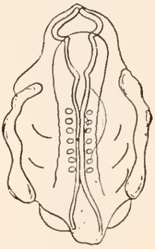
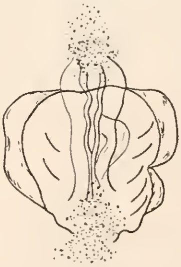
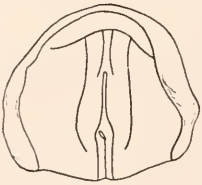
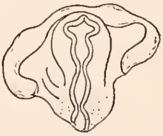
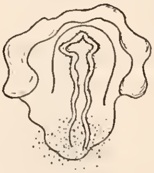
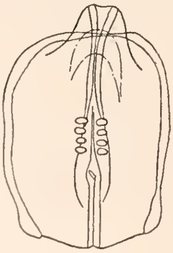
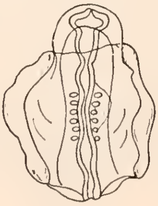
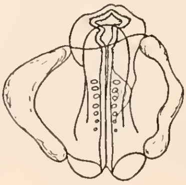
The results obtained are summarized in Table II. It is to be noted that $10^{-4} M$ iodoacetate produces almost immediate stoppage of development and complete degeneration, dispersal and disintegration of the entire embryo. The degree of inhibition at this concentration compared with the type of degeneration resulting from substrate deprivation (non-nutrient controls), indicates that the utilization of endogenous reserves as well as of exogenous substrates is blocked. At lower inhibitor concentrations ($5 \times 10^{-5} M$ and $2 \times 10^{-5} M$) the effects are less drastic and become differential (Table II). It is noteworthy that the time course and

---

[3] It is possible that the utilization of an excess amount of pyruvate might "throw out of gear" the entire metabolic mechanism by leading to the accumulation of excess endogenous metabolites such as ATP. Glucose might act as a phosphate acceptor and prevent its accumulation (Ochoa, 1941).

TABLE II

Monoiodoacetate

MEDIUM	NO. EXPL	STAGE EXPLANTED	GENERALIZED RESULTS	
			10± HOURS IN VITRO	20± HOURS IN VITRO
GLUCOSE 10^{-2}M + CH_2ICOOH 10^{-4}M	10			
GLUCOSE 10^{-2}M + CH_2ICOOH 5×10^{-5}M	26			
GLUCOSE 10^{-2}M + CH_2ICOOH 2×10^{-5}M	26			
PYRUVATE 2×10^{-2}M + CH_2ICOOH 10^{-4}M	9			
PYRUVATE 2×10^{-2}M + CH_2ICOOH 5×10^{-5}M	17			

spatial pattern of the differential degenerative process is almost identical with that occurring in the non-nutrient controls: the node and the head-fold, in this order, being the two most sensitive regions. In other words, actively differentiating regions seem to be particularly sensitive to the inhibitor. Eventually the entire neural axis (brain and cord) undergoes degeneration whereas the heart is relatively unaffected, continuing to develop and pulsate (Spratt, 1949a). The fact that the heart will develop in primitive streak blastoderms, in the presence of the inhibitor, argues against the possibility that the differential effect reflects primarily differences in the extent of development of the heart compared with the brain, *i.e.*, not only the maintenance but the initiation of heart-formation is more resistant than the brain. These differential effects on the processes of brain and heart-formation revealed by using the lower concentrations of iodoacetate suggest that the underlying metabolic processes are different.

Further elucidation of the apparently different metabolic mechanisms underlying brain-formation as compared with heart-formation would depend, partly, upon evidence as to the point of action of iodoacetate on the metabolic pathway.[4] The fact that both complete and differential inhibition of development by iodoacetate can be prevented by substitution of sodium pyruvate for glucose in the culture medium (Table II) suggests that the site of action of the inhibitor in the chick embryo is similar to that described for other organisms, namely, that it interferes with triosephosphate dehydrogenase. Partial protection against the effects of $10^{-4}M$ iodoacetate and complete protection against $5 \times 10^{-5}M$ iodoacetate are obtained with $2 \times 10^{-2}M$ pyruvate. The more extensive degeneration of the developing nervous system in the presence of glucose and iodoacetate (which presumably interferes with the important oxidative-phosphorylation reaction) [4] suggests its greater dependence upon oxidative metabolism as compared with the heart. Partial prevention, with pyruvate, of the complete degeneration produced by $10^{-4}M$ iodoacetate exhibits a differential pattern of degeneration which is correlated with the regional pattern of developmental activity (Table II).

(2) *Sodium fluoride.* The effects of this inhibitor and their prevention by substitution of pyruvate for glucose are summarized in Table III. Explants to a glucose-Ringer medium containing $10^{-2}M$ sodium fluoride undergo differential and then complete degeneration, the regional pattern of which is comparable to that resulting from substrate deprivation (Tables I and III). This is in contrast to $10^{-4}M$ iodoacetate which produces immediate and complete degeneration (Table II). Fluoride, at this concentration, apparently does not extensively inhibit the utilization of endogenous reserves.

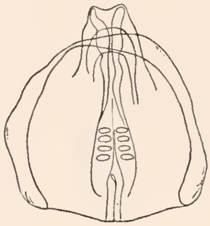
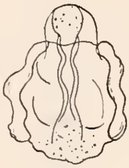
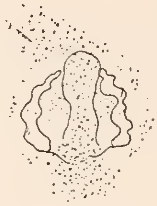
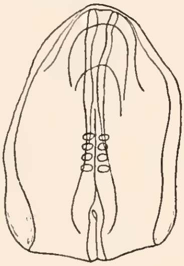
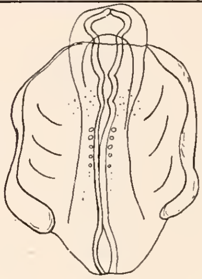
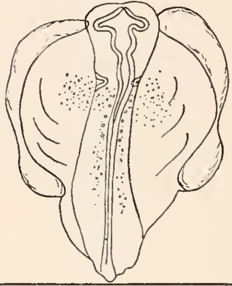
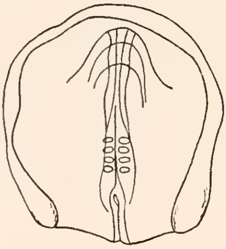
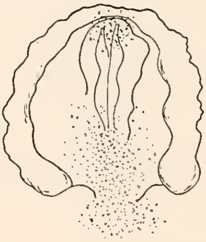
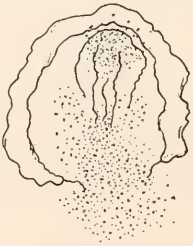
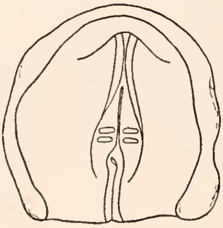
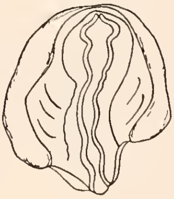
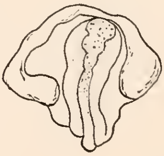
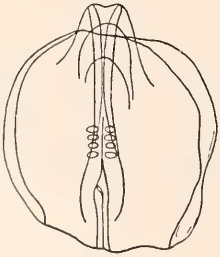
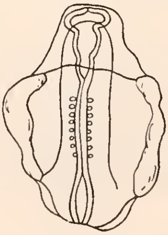
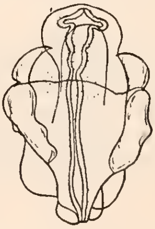
Lower concentrations of fluoride ($5 \times 10^{-3}M$) result in a differential inhibition of developmental processes which is almost the converse of that obtained with iodoacetate: The heart fails to develop or degenerates if already forming at the

---

[4] It is generally held that small amounts of iodoacetate (3×10^{-4} to $3 \times 10^{-3}M$) inhibit triosephosphate dehydrogenase, the enzyme catalyzing the reaction: 1, 3 Diphosphoglyceraldehyde $\rightleftarrows$ 1,3 Diphosphoglyceric acid (Dixon, 1937; Adler, v. Euler and Günther, 1938; Rapkine, 1933 and 1938). Originally it was thought that iodoacetate completely blocked anaerobic glycolysis in concentrations which had little or no effect on respiration (Lunsgard, 1930) but subsequently it has been shown that these concentrations ($3 \times 10^{-4}M$) also markedly depress respiration in rat brain and testis (Krebs, 1931; Fuhrman and Field, 1943).

Table III

Sodium fluoride

MEDIUM	NO. EXPL	STAGE EXPLANTED	GENERALIZED RESULTS	
			10 ± HOURS IN VITRO	20 ± HOURS IN VITRO
GLUCOSE 10^{-2}M + FLUORIDE 10^{-2}M	37			
GLUCOSE 10^{-2}M + FLUORIDE 5×10^{-3}M	44			
PYRUVATE 2×10^{-2}M + FLUORIDE 2×10^{-2}M	8			
PYRUVATE 10^{-2}M + FLUORIDE 10^{-2} M	7			
PYRUVATE 2×10^{-2}M + FLUORIDE 10^{-2} M	16			

time of explantation, whereas the central nervous system continues its development and shows little if any harmful effect of the fluoride. At lower fluoride concentrations (2.5×10^{-3} and $10^{-3}M$) there is progressively less effect upon heart-formation. The typical picture of the differential effect is that of an apparently normal embryo with a mass of dispersed and degenerating mesodermal cells occupying the position in which the heart would have formed. The degenerative effect of fluoride is not limited to heart mesoderm but involves, to some extent, the somite and lateral plate mesoderm. In a number of cases, posterior development of the neural tube is indirectly inhibited, presumably by the absence of inducing mesoderm. Some of these effects are illustrated in Figure 1.

Interpretation of the differential effects of fluoride, in terms of interference with the metabolic mechanisms underlying heart as compared with brain-formation, is largely dependent upon knowledge of its site of action.[5] The fact that complete inhibition of development by $10^{-2}M$ fluoride can be prevented by substitution of a sufficient amount ($2 \times 10^{-2}M$) of pyruvate for glucose (Table III) suggests that the inhibitor is blocking enolase activity. If this is true, the greater sensitivity of the heart as compared with the developing brain would indicate its greater dependence upon a glycolytic energy source. Since the effect of fluoride on respiration is relatively indirect and slight (see Gemmill, 1939, for discussion of the mechanism of fluoride inhibition) compared with the effect of iodoacetate, one would expect that its interference with the metabolic basis of brain-formation would be correspondingly less drastic. Eventually, the piling up of metabolic intermediates in the presence of fluoride would presumably interfere with the glycolytic and oxidative pathways above pyruvate. Indeed, fluoride does have a delayed inhibitory effect upon the nervous system during the second day of cultivation (Fig. 1).

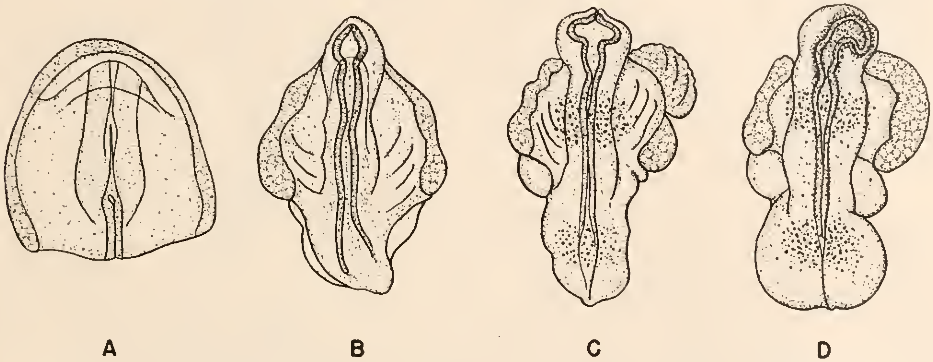


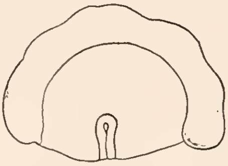
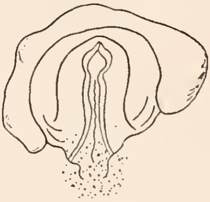
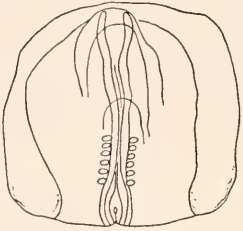
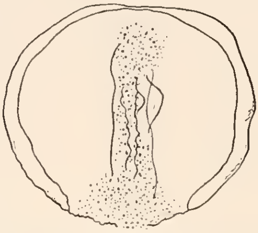
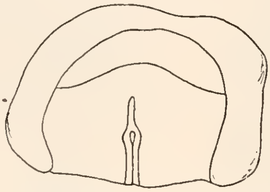
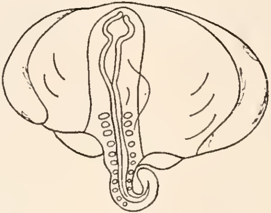
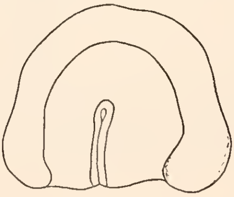
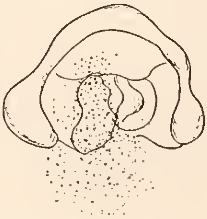
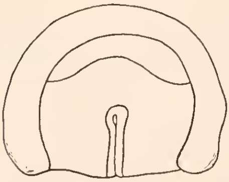
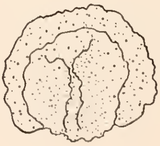
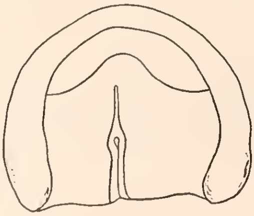
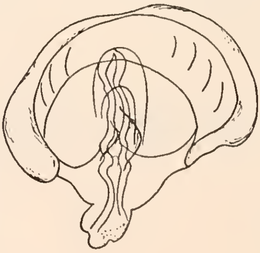
FIGURE 1. Camera lucida drawings of the anterior half of a living explant during its development on a medium containing Ringer, $10^{-2}M$ glucose, and $5 \times 10^{-3}M$ sodium fluoride. A. Stage explanted; B. 8 hours later; C. 18 hours after explantation; D. 38 hours after explantation. Note the degenerating heart mesoderm.

---

[5] It is generally held that fluoride inhibits the enzyme enolase which catalyzes the glycolytic reaction: 2-Phosphoglyceric acid $\rightleftarrows$ Phosphoenolpyruvic acid, by formation of a magesium-fluoride phosphate complex (Meyerhof and Kiessling, 1935; Warburg and Christian, 1942).

TABLE IV

Citrate, malonate, cyanide, azide
($10^{-2}M$ glucose present in all media)

INHIBITOR	NO. EXPL	STAGE EXPLANTED	GENERALIZED RESULTS
			$20^{\pm}$ HOURS IN VITRO
CITRATE 5×10^{-3}M	29		
MALONATE 10^{-3} M	38		
CYANIDE 5×10^{-3}M	13		
CYANIDE 10^{-2}M	12		
AZIDE 5×10^{-4}M	6		
AZIDE 10^{-4}M	6		

(3) Sodium citrate. Not only is the early embryo unable to utilize citrate as a carbon source but its utilization of glucose is partially inhibited by 5×10^{-3} to $10^{-2}M$ concentrations of sodium citrate (Table IV). It was thought that citrate would produce an inhibitory effect like that of fluoride because of the formation of relatively insoluble magnesium citrate which would displace magnesium, the normal activator of enolase. However, since there are several other enzymes which depend upon magnesium as an activator, only rather general inhibitory effects could be expected. In fact, citrate produces a pattern of differential inhibition similar to that obtained with iodoacetate, malonate, cyanide and azide; development of the central nervous system is inhibited, but not that of the heart.

(4) Sodium malonate. The effects of $10^{-3}M$ sodium malonate are summarized in Table IV. The differential effects produced by this and by twice this concentration are striking; usually no effect of the inhibitor is observed during the first 20 hours *in vitro*. During the next 20 hour period, the central nervous system degenerates completely. Meanwhile the heart forms and functions. The differential effect of malonate is of the same type as that produced by iodoacetate but is still more pronounced, an interesting observation since malonate is a rather specific inhibitor of succinic dehydrogenase (Quastel and Wooldridge, 1928; Ochoa, 1944) in the cyclophorase system and would not be expected to interfere directly with the glycolysis of glucose by the heart. Iodoacetate would presumably not produce as clear cut a separation of anaerobic glycolysis and respiration (see above).

If the effect of malonate on the developing embryo is the result of its competitive inhibition of succinic dehydrogenase, the addition of succinate to the medium should diminish or prevent the effect. Preliminary experiments in which both $2 \times 10^{-3}M$ malonate and $10^{-3}M$ succinate are present in the medium indicate a partial prevention of the malonate inhibition. Greater concentrations of succinate ($4 \times 10^{-3}M$) are more effective. Preliminary incubation for 6 hours in the presence of glucose and $4 \times 10^{-3}M$ succinate, followed by subculture to a medium containing glucose, malonate ($2 \times 10^{-3}M$) and succinate ($4 \times 10^{-3}M$), results in the best (incomplete) protection against the malonate effect. Attempts to by-pass the inhibited step with sodium fumarate have failed, presumably because exogenous fumarate cannot be utilized by the embryo (Spratt, 1949b and 1950).

(5) Sodium cyanide. Since it had previously been shown that oxygen deficiency has a more pronounced effect on development of the central nervous system than on the heart (Spratt, 1948b and 1950) it was of some interest to study the effect of the respiratory inhibitors, cyanide and azide, both of which according to Keilin and Hartree (1939) act on cytochrome oxidase. Reference to Table IV shows that $5 \times 10^{-3}M$ sodium cyanide in the medium (pH 7.8–8.5) has no visible effect on development. Higher concentrations ($10^{-2}M$) give the typical pattern of differential inhibition as regards the time course and spatial pattern of the degenerative process. As would be expected, the developing brain is more sensitive to cyanide than is the heart. This result is comparable with the effects of cyanide on the squid embryo (Marvel and Fisher, 1948).

(6) Sodium azide. Relatively low concentrations of sodium azide ($5 \times 10^{-4}M$) bring about immediate and complete degeneration and disintegration of the explants

(Table IV). As was the case with monoiodoacetate (see above) this concentration of azide apparently blocks the utilization of endogenous as well as exogenous substrates.[6]

Lower concentrations of azide ($10^{-4}M$) are less toxic and tend to produce a pattern of differential degeneration of the same type as that produced by iodoacetate and malonate, but never as distinct. Indeed, the results to date have shown no clear cut differential effects of azide: concentrations which are sufficiently high to bring about degeneration of the central nervous system also seem to inhibit heart-formation; those just low enough to permit heart development result in abnormal but not inhibited brain development (Table IV).

The absence of distinct differential effects of sodium azide is interesting in the light of its action as an inhibitor of synthetic processes (Clifton, 1937; Meyerhof, 1945). In the frog, azide (in contrast to cyanide) also brings about an immediate cessation of development at all stages studied (Spiegelman and Moog, 1945). Whereas some developmental changes, *e.g.*, heart-formation, can occur under partial anaerobiosis (low oxygen tension or cyanide) these cannot occur in the presence of azide, apparently, because of its additional effect upon the coupling between energy yielding and energy requiring reactions. Evidence for such an action of azide is found in the studies of Barth and Jaeger (1947) on phosphorylation in the frog's egg.

(7) Interpretation of differential effects of monoiodoacetate and sodium fluoride. The observation that mesodermal derivatives are more sensitive to fluoride than are ectodermal derivatives, together with the converse observation that ectodermal derivatives are more sensitive to monoiodoacetate than are mesodermal derivatives, indicates differences in the metabolic processes underlying the formation and maintenance of the two different germ layer derivatives. Although the exact nature of the biochemical differences is not revealed by the above observations, the following explanation is offered as a working hypothesis:

a. It has been shown that the brain is more sensitive to oxygen deficiency than is the heart (Spratt, 1948b and 1950).

b. The brain is also more sensitive to cyanide and azide, substances which interfer with cytochrome oxidase activity.

c. The brain is more sensitive to iodoacetate but the heart is more sensitive to fluoride.

d. The interpretation that the concentration of iodoacetate used ($2 \times 10^{-5}M$) is partially blocking both anaerobic glycolysis and respiration (Krebs, 1931; Fuhrman and Field, 1943), and the concentration of fluoride used ($5 \times 10^{-3}M$) is primarily inhibiting glycolysis, is consistent with the hypothesis that the brain depends primarily upon oxidative metabolism, the heart upon glycolysis.

---

[6] The similarity in the effects of these two inhibitors is interesting in view of the evidence (Spiegelman, Kamen and Sussman, 1948) that azide not only inhibits the Warburg-Keilin System but also the coupling mechanism between oxidation and phosphate esterification in the reaction: 3-Phosphoglyceraldehyde $\leftrightarrows$ 1,3-Diphosphoglyceric acid, *i.e.*, the diphosphoglycerate-transphosphorylase system close to the site of action of iodoacetate.

DISCUSSION

Changes in the environment of the explanted, early embryo whether consisting of (a) the presence of metabolic inhibitors, (b) substrate deprivation (Spratt, 1949a and 1950), (c) partial anaerobiosis (Spratt, 1948b), (d) carbon dioxide deficiency (Spratt, 1949c) or (e) low pH of the medium (Spratt, 1950) all lead to a characteristic pattern of differential inhibition of development, the time course and spatial features of which are essentially identical with the axial gradient pattern of physiological activity of the blastoderm (Child, 1925; Hyman, 1927; Rulon, 1935). The common effects of these diverse environmental modifications are summarized diagrammatically in Figure 2 in terms of regional and gradient differences in sensitivity (density of stippling) of A, head-fold and B, 10-somite blastoderms. Centers of developmental activity (morphogenesis and differentiation) at the two stages are indicated by the symbols X and Y, and the specific developmental events are noted at the side. The arrows indicate the time course (spread) of the degeneration.

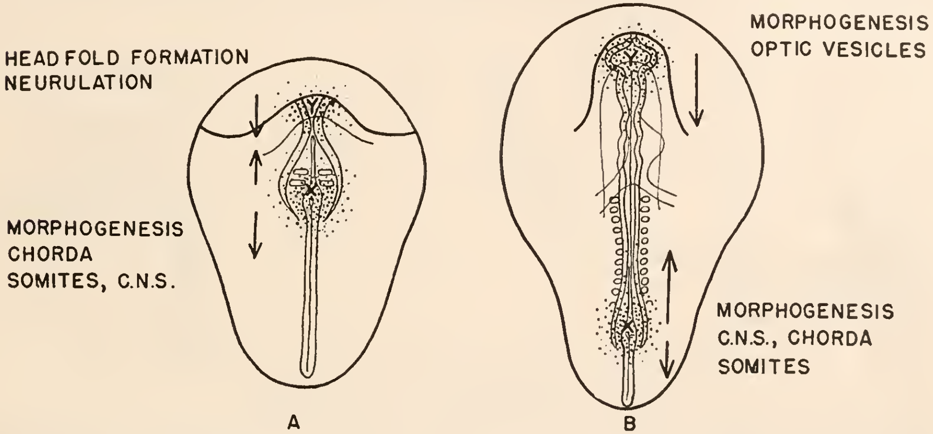


FIGURE 2. Diagrams summarizing the time course and spatial features of the characteristic pattern of differential inhibition of development produced by inhibitors, starvation, partial anaerobiosis, etc. Note that this pattern is correlated with that of differentiation activity. See text for further descripion.

All of the environmental modifications have one primary effect in common: they interfere with the utilization of exogenous substrates upon which the embryo is dependent. In other words, they all retard or inhibit the metabolic processes underlying developmental activities. Furthermore, they all reveal both qualitative and quantitative differences in the nutritional requirements for formation and maintenance of different organs and tissues (Spratt, 1950). On the other hand, each environmental modification, in general, is like the others in demonstrating that the nutritional requirements for the *maintenance* of a differentiated structure (*e.g.*, brain, heart, etc.) are qualitatively the same as those for its *formation* (differentiation). These observations, together with the results of the inhibitor experiments in particular, indicate that there is no qualitative difference between the metabolic basis (at least as regards the fundamental energy-yielding mechanisms) of the differentiation as compared with the maintenance of a structure. The theoretical implications of this concept of the "identity of maintenance and morphogenetic energy" have been discussed by Spiegelman (1945) in connection with his physiological competition hypothesis.

Finally, analysis of the inhibitor and previous experiments on differential nutrient requirements, in so far as they reveal a pattern of both quantitative and qualitative differences in metabolic activity in the early embryo, demonstrates that there is an apparently significant coincidence between the time course and spatial pattern of degeneration produced by inhibitors or starvation and the time course and spatial pattern of differentiation activity (Fig. 2). Regions of the early embryo where differentiation activity is greater (*e.g.*, the node and head-fold, or later, the fore-brain and the segmental plate, etc.) are more sensitive to adverse environmental conditions (presence of metabolic inhibitors, starvation, oxygen deficiency) than are regions which have already passed through a period of differentiation activity (*e.g.*, hind-brain, spinal cord, somites, pulsating heart, etc.). It is thus not surprising that the energy requirements (in terms of substrate level necessary) for morphogenesis and histogenesis are greater than are those for maintenance. The building up of a new level of structural complexity which characterizes the form-building and tissue-building aspects of differentiation would obviously involve the expenditure of more energy than that needed to maintain the preceeding level of complexity against the ever present tendency toward "structural death."

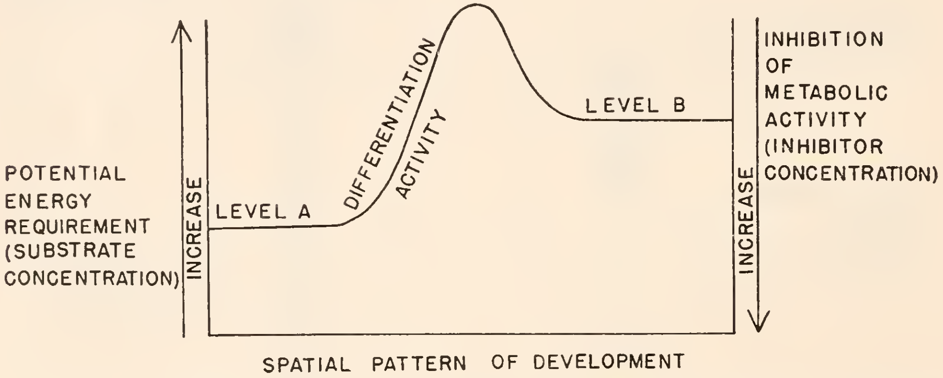

POTENTIAL ENERGY REQUIREMENT (SUBSTRATE CONCENTRATION)
INCREASE
LEVEL A
DIFFERENTIATION ACTIVITY
LEVEL B
INCREASE
INHIBITION OF METABOLIC ACTIVITY (INHIBITOR CONCENTRATION)
SPATIAL PATTERN OF DEVELOPMENT

FIGURE 3. Diagram illustrating the relationship of substrate requirements and inhibitor effects to differentiation activity and to a spatial pattern of development. See text for further description.

A more thorough examination of the data pertaining to the differential effects of substrate deprivation, inhibitors, etc. reveals the extremely interesting fact that the energy necessary for the maintenance of an actively differentiating region, transforming from one level of structural complexity to the next, is *more* than the sum of the energy necessary for maintenance of each level. Thus, about 5 mg. glucose, 10 mg. fructose, or 20 mg. galactose (per 100 ml. medium) will maintain relatively undifferentiated regions; whereas, roughly 10 mg. glucose, 20 mg. fructose or 100 mg. galactose will prevent degeneration of differentiated regions; but 15–20 mg. glucose, 50 mg. fructose or 200 + mg. galactose are necessary to maintain actively differentiating regions (*e.g.*, the node or fore-brain). Although the quantities as stated are only approximations, they are comparatively significant (Spratt, 1949a, pp. 286–291 and 1950). This extra energy requirement may be grossly compared with the "activation-energy" of a chemical reaction except, of course, that there is an *increase* in free energy of the system. In Figure 3 these relations are diagrammatically represented.

For example, as the substrate concentration in the medium is increased it will first satisfy the energy requirements of relatively undifferentiated regions, Level A (*e.g.*, extra-embryonic region compared with embryonic area, spinal cord compared with brain, etc.) and other parts of the blastoderm (both differentiating and differentiated regions) will degenerate and undergo cell dispersal. Further increase would maintain structural Level B, but not the region undergoing the transformation, A → B (*e.g.*, the node). Inhibition of metabolic activity has the converse effect. In terms of synthetic activities, the change from A → B is an increase in rate, surpassing even the rate required to maintain Level B. The greater substrate requirement and sensitivity to inhibitors of an embryonic region undergoing active transformation (*e.g.*, the node) may be compared with its greater differentiation potential[7] and array of tissue-forming potencies. It is tempting to speculate that the structural complexity of such a region is *less* than that of either a relatively undifferentiated or differentiated region.

In conclusion, it would be interesting to interpret the above relations in terms of the underlying enzymatic activities. As an hypothesis, it is suggested that enzymatic activity is inversely proportional to the level of structural differentiation or, conversely, directly proportional to the nutritional (potential energy) requirements of the embryonic region. During the differentiation process (Fig. 3) there would first be an increase in enzyme activity (associated with increased synthesis) followed by a decrease to a new level which, however, is higher than that preceeding the transformation process. The increase followed by a decrease in activity might be proportional to the release from, followed by the incorporation of enzyme proteins into, the structural organization of the cell or cell groups. It is interesting that such rapidly transforming regions as the node and fore-brain also exhibit the greatest capacity for regulation and regeneration (Waddington, 1932; Spratt, 1940). These regions, in contrast to others, might have more "free" enzymes available for the additional catalyses necessary to replace a lost part. Unpublished studies on the reduction of vital dyes, some of the studies of Child and his students, and studies of specific dehydrogenase activities now in progress at least suggest the plausibility of such a concept.

SUMMARY

1. The effects of metabolic inhibitors on the development *in vitro* of approximately 600 chick blastoderms (definitive primitive streak through 8-somite stages) have been studied.

---

[7] Used in the sense as defined and described by Spiegelman (1945).

2. Embryos explanted to glucose media containing either 10^{-4} to $5 \times 10^{-5}M$ monoiodoacetate or $10^{-2}M$ fluoride rapidly undergo complete degeneration and disintegration. These effects are reversible by substitution of $2 \times 10^{-2}M$ pyruvate (or lactate) for the glucose. At lower inhibitor concentrations of iodoacetate ($2 \times 10^{-5}M$) the central nervous system degenerates or fails to form, but the heart develops and pulsates. Fluoride has almost the opposite effect: concentrations which cause degeneration of the heart ($5 \times 10^{-3}M$) have no appreciable effect upon the developing central nervous system.

3. Other inhibitors: citrate, malonate, cyanide and azide, produce a differential pattern of inhibition similar to that produced by iodoacetate. Azide, however, does not lead to as clear cut a pattern of differential degeneration as do the others.

4. Comparison of the effects of inhibitors with those produced by substrate deprivation, low oxygen tension, etc. reveals that all of these environmental modifications give rise to a characteristic pattern of differential degeneration in the blastoderm, the time course and spatial pattern of which is essentially identical with that occurring in the non-nutrient control series; the node and the headfold, in this order, being the two most sensitive regions.

5. The pattern of differential inhibition of development produced by the presence of inhibitors or other environmental modifications coincides with the pattern of differentiation activity and, presumably, with the pattern of underlying metabolic (enzymatic) activity.

6. Analysis of the experimental results has led to the formulation of a general hypothesis relating developmental activity, energy requirements and enzyme activity to the gradual transformation from one level of structural complexity to the next.

LITERATURE CITED

Adler, E., H. v. Euler and G. Günther, 1938. Dehydrasen und Jodessigsäure. *Skand. Arch. Physiol.*, **80**: 1–15.

Barth, L. and L. Jaeger, 1947. Phosphorylation in the frog's egg. *Physiol. Zool.*, **20**: 133–146.

Child, C., 1925. The physiological significance of the cephalocaudal differential in vertebrate development. *Anat. Rec.*, **31**: 369–383.

Clifton, C., 1937. On the possibility of preventing assimilation in respiring cells. *Enzymologia*, **4**: 246–253.

Dixon, M., 1937. Action of iodoacetate on dehydrogenases and alcoholic fermentation. *Nature*, **140**: 806.

Fuhrman, F. and J. Field, 1943. Effect of iodoacetate on respiration and glycolysis in excised rat brain. *J. Cell. Comp. Physiol.*, **21**: 307–317.

Gemmill, C., 1939. The inhibition of glycolysis. *Cold Spring Harbor Symp.*, **7**: 216–226.

Hyman, L., 1927. The metabolic gradients of vertebrate embryos. III. The chick. *Biol. Bull.*, **52**: 1–38.

Keilin, D. and E. Hartree, 1939. Cytochrome and cytochrome oxidase. *Proc. Roy. Soc.*, (B), **127**: 167–191.

Krebs, H., 1931. Über die Wirkung der Monojodessigsäure auf den Zellstoffwechsel. *Biochem. Zeit.*, **234**: 278–282.

Lunsgard, E., 1930a. Die Monojodessigsäurewirkung auf die enzymatische Kohlenhydratspaltung. *Biochem. Zeit.*, **220**: 1–7.

Lunsgard, E., 1930b. Über die Einwirkung der Monojodessigsäure auf den Spaltungs—und Oxydationsstoffwechsel. *Biochem. Zeit.*, **220**: 8–18.

Marvel, R. and K. Fisher, 1948. Developmental changes in the viability of squid embryos after subjection to cyanide. *Biol. Bull.*, **94**: 45–54.

Meyerhof, O., 1945. The origin of the reaction of Harden and Young in cell-free alcoholic fermentation. *J. Biol. Chem.*, **157**: 105–119.

Meyerhof, O. and W. Kiessling, 1935. Über den enzymatischen Umsatz der synthetischen Phosphobrenztraubensäure (enol-Brenztraubensäure-phosphorsäure). *Biochem. Zeit.*, **280**: 99–109.

Ochoa, S., 1941. Glycolysis and phosphorylation in brain extracts. *J. Biol. Chem.*, **141**: 245–251.

Ochoa, S., 1944. a-Ketoglutaric dehydrogenase of animal tissues. *J. Biol Chem.*, **155**: 87–100.

Quastel, J. and W. Wooldridge, 1928. Some properties of the dehydrogenating enzymes of bacteria. *Biochem. J.*, 22: 689–702.

Rapkine, L., 1933. Sur l'action de l'acide monoiodoacétique sur les groupments sulphydrilés liés aux protéines. *Compt. Rend. Soc. Biol.*, **112**: 1294–1296.

Rapkine, L., 1938. Sulphydryl groups and enzymic oxido-reduction. *Biochem. J.*, **32**: 1729–1739.

Rulon, O., 1935. Differential reduction of Janus green during development of the chick. *Protoplasma*, **24**: 346–364.

Spiegelman, S., 1945. Physiological competition as a regulatory mechanism in morphogenesis. *Quart. Rev. Biol.*, **20**: 121–146.

Spiegelman, S., M. Kamen and M. Sussman, 1948. Phosphate metabolism and the dissociation of anaerobic glycolysis from synthesis in the presence of sodium azide. *Arch. Biochem.*, **18**: 409–436.

Spiegelman, S. and F. Moog, 1945. A comparison of the effects of cyanide and azide on the development of frog's eggs. *Biol. Bull.*, **89**: 122–130.

Spratt, N., Jr., 1940. An *in vitro* analysis of the organization of the eye-forming area in the early chick blastoderm. *J. Exp. Zool.*, **85**: 171–209.

Spratt, N., Jr., 1947. Development *in vitro* of the early chick blastoderm explanted on yolk and albumen extract saline-agar substrate. *J. Exp. Zool.*, **106**: 345–365.

Spratt, N., Jr., 1948a. Development of the early chick blastoderm on synthetic media. *J. Exp. Zool.*, **107**: 39–64.

Spratt, N., Jr., 1948b. Nutritional requirements of the early chick embryo. II. Differential nutrient requirements for morphogenesis and differentiation of the heart and brain. *Anat. Rec.*, **101**: 716.

Spratt, N., Jr., 1949a. Nutritional requirements of the early chick embryo. I. The utilization of carbohydrate substrates. *J. Exp. Zool.*, **110**: 273–298.

Spratt, N., Jr., 1949b. Nutritional requirements of the early chick embryo. III. The metabolic basis of morphogenesis and differentiation as revealed by the use of inhibitors. *Anat. Rec.*, **105**: 117.

Spratt, N., Jr., 1949c. Carbon dioxide requirements of the early chick embryo. *Anat. Rec.*, **105**: 118.

Spratt, N., Jr., 1950. Nutritional requirements of the early chick embryo. II. Differential nutrient requirements for morphogenesis and differentiation of the heart and brain. *J. Exp. Zool.* (in press).

Waddington, C., 1932. Experiments on the development of chick and duck embryos, cultivated *in vitro*. *Philos. Trans. Roy. Soc.*, B., **221**: 179–230.

Warburg, O. and W. Christian, 1942. Isolierung und Kristallisation des Gärungsferments Enolase. *Biochem. Zeit.*, **310**: 384–421.

FURTHER OBSERVATIONS ON CERCARIA PARVICAUDATA STUNKARD AND SHAW, 1931

HORACE W. STUNKARD

New York University

In a report on the effect of dilution of sea-water on the activity and longevity of certain marine cercariae, Stunkard and Shaw (1931) described two new species of cercariae. One of them, *Cercaria sensifera*, was shown by Stunkard and Cable (1932) to be the larval stage of *Parorchis avitus* Linton, 1914 [= *P. acanthus* (Nicoll, 1906) Nicoll, 1907]. The life history and adult stages of the other species, *Cercaria parvicaudata*, is yet unknown. The original account of *C. parvicaudata* was brief, incomplete and in certain respects inaccurate; further study permits a more adequate description of the species and may aid in the elucidation of the life cycle.

Cercaria parvicaudata occurs in *Littorina littorea* (Linn., 1758) Férussac, 1822; in *L. saxatilis* (Olivi, 1792) Johnston, 1841 [= *L. rudis* (Maton, 1797) Forbes, 1838]; and in *L. obtusata* (Linn., 1758) Férussac, 1822 [= *L. palliata* (Say, 1822)].[1] So far, the infection has been reported only in the region of Woods Hole, Mass. About one per cent of the individuals of the first two species is infected, but infections in *L. obtusata* are rare. Shedding of the cercariae is irregular; often an infected snail will not shed any cercariae for several days. The larvae may emerge at night or during the day. Multiple infections with *Cercaria parvicaudata* and other species, *viz.*, cercariae of *Cryptocotyle lingua*, and with a small undescribed microphallid species, have been found in *L. littorea* and in *L. saxatilis*.

The cercariae are produced in orange-colored sporocysts, localized usually in a single, oval mass situated between the intestine and the body wall of the snail and about midway between the ends of the visceral portion. The mass may be from 2 to 10 mm. long; when large it causes protrusion of the body wall and is easily recognized by the orange color when the shell of the snail is removed. Occasionally more than one mass is present and as many as four have been observed. Each mass contains from hundreds to thousands of daughter sporocysts restricted to a circumscribed region; they do not occur in the lymph spaces throughout the body of the snail and are absent from the digestive gland, although cercariae have been found in the hemocoele when snails were dissected. It is probable that each mass of sporocysts constitutes the progeny of a single miracidium, and when two or more masses are present, which is rare, that multiple infection has occurred. The location of the sporocysts suggests that the snail ingests the embryonated egg of the trematode, that the miracidium emerges in the intestine, migrates through the gut wall and transforms into a primary sporocyst in which asexual reproduction produces the mass of daughter sporocysts.

---

[1] Synonymy after Bequaert (1943), "The Genus *Littorina* in the Western Atlantic." In Johnsonia; No. 7, 28 pp., Museum Comp. Zool., Harvard University.

A half-grown specimen of *L. littorea* infected with *C. parvicaudata* and a somewhat more mature specimen of *L. saxatilis* infected with both *C. parvicaudata* and the undescribed microphallid species were killed, fixed, and cut in serial sections, 15 microns thick. Sections were stained with Ehrlich's haematoxylin and erythrosin. The specimen of *L. littorea* had been isolated for 23 days in a finger bowl. Early in the period it had shed cercariae in considerable numbers, several hundred in 24 hours, but the number gradually diminished and in the last week no cercariae were observed. Perhaps lack of food had so depleted the nutrient material in the blood of the snail that development and liberation of cercariae had stopped. One mass of sporocysts was present; at that level the intestine was pressed against the body wall and all the space to the opposite body wall was filled with sporocysts; in one section 286 sporocysts were counted. The lobes of the digestive gland were almost completely excluded from this portion of the snail and no gonad was recognized. In much of the mass the sporocysts were almost contiguous, but, near the periphery especially, were separated by loose connective tissue of the snail. It is clear that the sporocysts do not migrate and none was found in any other part of the snail. No free cercariae were found in the hemocoele.

The snail had been isolated for three weeks in the narrow confines of a finger bowl and during this time had liberated thousands of cercariae. Sections revealed hundreds of encysted metacercariae, located under the body wall and in the mantle, tentacles and edges of the foot. The cysts measured 0.1–0.12 mm. in diameter; the metacercariae were identical morphologically with the cercariae of *C. parvicaudata* and undoubtedly had been produced in this snail. It would appear, therefore, that infection with the asexual stages of the parasite does not confer immunity against subsequent invasion by cercariae.

The sporocysts (Fig. 7), which are undoubtedly second generation or daughter sporocysts, are oval to pyriform to clavate sacs, 0.3–0.9 mm. long and 0.15–0.35 mm. wide. There is a birth pore at one end of the sporocyst, visible only when a cercaria is emerging, and the cavity contains germinal cells, germ balls of various sizes and developing cercariae; sporocysts within sporocysts have not been observed. The germinal material, whether as single cells or as multicellular masses, always stains intensely and appears to be free in the cavity. In living sporocysts, the germinal elements are pushed about by movements of the young cercariae. The wall of the sporocyst consists of two layers: an inner layer of flattened cells in which the nuclei are clear and stain well, and an outer layer, the paletot, of large pigment bearing cells in which the nuclei are indistinct and do not stain. When the sporocyst is extended the lateral walls are thin and each layer is one cell in thickness, but if the sporocyst is contracted the cells may be compressed and superposed. At the ends of the sporocyst, especially the end where the birth pore is located, each layer of the wall may be several cells in thickness. The external ends of the cells of the paletot form a pebbled or beaded surface which is closely invested by a layer of flattened cells that are part of and continuous with the connective tissue of the snail. In the wall of the sporocyst there are scattered circular and longitudinal fibers, but living sporocysts have little or no motility when freed in sea-water.

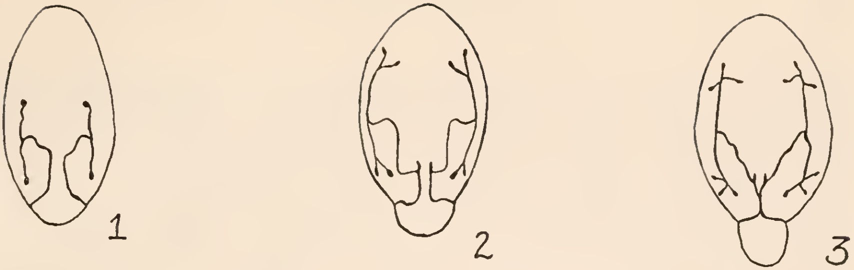
The cercariae (Fig. 4) are oval to ovate from dorsoventral aspect and the wider portion may be either anterior or posterior to the middle of the body. Mature cercariae measure 0.14–0.36 mm. in length as the body is contracted or extended, and the width varies inversely with the length, measuring 0.05–0.12 mm. The body, but not the tail, is covered with minute, sharp-pointed spines, deeply embedded in the cuticula. The dorsal wall of the oral sucker bears a simple stylet (Fig. 5), 0.015 mm. long and 0.0032 mm. wide. There are small dorsal and ventral flanges on the stylet at the base of the conical tip. The tail is subterminal in attachment and is very active; it may be contracted to a length of 0.06 mm. or extended until it exceeds the length of the body. When the worm is attached or creeping or immobilized under a coverglass, the tail manifests nervous, twitchy movements. In swimming, the body is contracted into a short, wide form and bent ventrally while the tail is extended and lashes vigorously. The tail is almost circular in cross section and the terminal one-third is narrowed slightly. The larvae swim at random in all levels of the water and are more or less uniformly distributed in a finger bowl. They do not respond to light or move in any particular direction except upward; indeed, the swimming movements are weak and the lashing of the tail merely keeps them suspended in the water. Although they progress very little as a result of their own activity, they are carried about by currents in the water. An acetabulum is present and may protrude; it is median slightly posterior to the middle of the body, 0.03–0.05 mm. in diameter and is surrounded by a few small papillae and 68–72 spines. Under very slight pressure the sucker measures about 0.04 mm. in diameter, but becomes larger as the larva is flattened. The mouth opening is subterminal; the oral sucker is spherical to oval, 0.035–0.06 mm. in diameter and the dorsal lip bears 18 minute spines. The oral sucker is followed almost immediately by the pharynx which measures about 0.014 mm. long and 0.012 mm. wide and the esophagus extends about one-half the distance to the acetabulum. The digestive ceca end near the level of the caudal margin of the acetabulum.

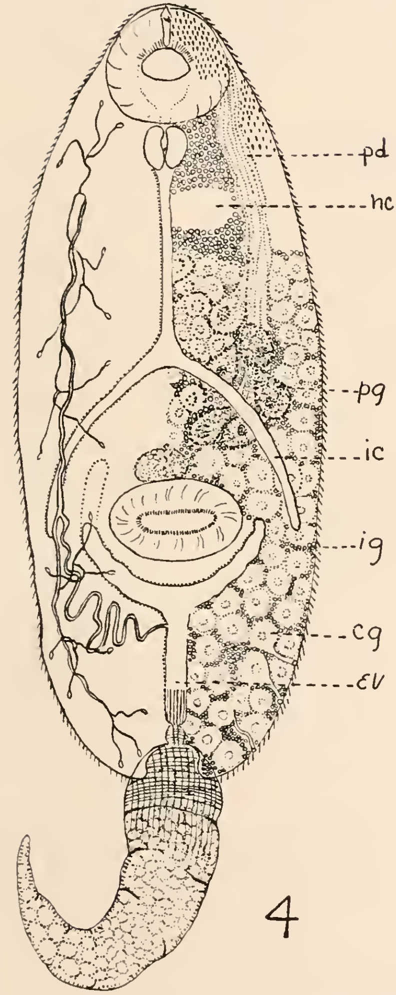
The body is largely filled with glandular cells. There are six pairs of penetration glands, situated in the acetabular and preacetabular areas. These cells have a finely-granular cytoplasm which is colorless in transmitted light and stains a rose-pink with neutral red. They are irregularly flask-shaped, often lobed, 0.011–0.018 mm. in diameter, and the nuclei are 0.005–0.006 mm. in diameter. Ducts from these cells pass forward and open at the sides of the stylet. Under pressure, the contents of the glands stream forward at different rates and may cause enlargements of the ducts, especially at or near the oral sucker. Behind the nerve commissure, which spans the esophagus dorsal and immediately posterior to the pharynx, the body contains about one-hundred cystogenous cells. They are

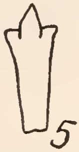
---

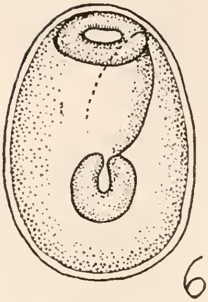
ABBREVIATIONS

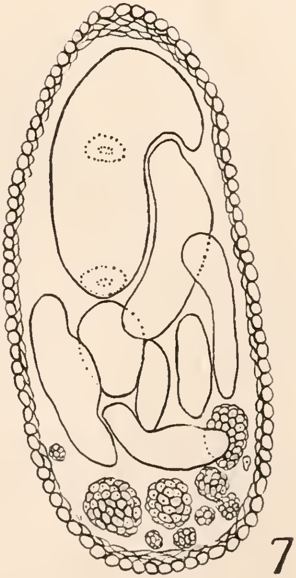
cg—cystogenous gland
ev—excretory vesicle
ic—intestinal cecum
ig—intercellular granules
nc—nerve commissure
pd—penetration gland duct
pg—penetration gland











FIGURES 1, 2, 3. Development of excretory system in developing cercariae.

FIGURE 4. Mature cercaria, excretory system shown on the left, other structures on the right side.

FIGURE 5. Stylet, lateral view.

FIGURE 6. Metacercaria from *L. littorea.*

FIGURE 7. Sporocyst with developing cercariae.

about the size and shape of the penetration glands, but the cytoplasm is flaky in appearance, yellowish-green in transmitted light and reddish-brown when stained with neutral red. Short ducts from the cystogenous glands lead to the surface of the body and under pressure the contents are extruded as minute droplets. The intercellular spaces of the parenchyma are filled with thousands of small, refringent spherules, 0.0015–0.0025 mm. in diameter. When extruded in sea-water, these droplets adhere and coalesce; they are lighter than water and lie against the under side of the coverglass. They are soluble in alcohol, less soluble in xylol, and insoluble in N/10 acids or alkalies. They may be excretory products, unsaturated fatty materials, produced by the reduction of carbohydrates. For a discussion of these substances, the reader is referred to the paper by Stephenson (1947).

The development of the excretory system has been traced in young cercariae, removed from sporocysts. In large germ balls there are two excretory pores and a doubled excretory system. The number of flame cells increases as shown in Figures 1, 2 and 3. Later in development the posterior portions of the two collecting tubules fuse to form the median excretory vesicle and the constriction which cuts off the tail is situated immediately anterior to the excretory pores. Accordingly, in young cercariae there are two short ducts from the vesicle and two excretory pores; later the two ducts fuse and when the tail is shed, the excretory pore is terminal. It is to be noted, before the fusion of the collecting ducts of the two sides, that there may be a small anterior evagination on each collecting duct immediately anterior to the point where it later fuses with its counterpart of the opposite side, Figure 2. These evaginations produce the anterolateral arms of the future excretory vesicle. In the mature cercaria, the vesicle is Y-shaped and ventral in position; anterior to the pore there is a strong sphincter and the median stem extends more than half way to the acetabulum. The lateral branches extend forward on either side of the acetabulum, but the wall of the vesicle is weak, the shape is inconstant and varies with the amount and location of the contained fluid. The vesicle collapses when empty and the anterior portions of the lateral branches disappear. Collecting ducts, long and very much convoluted, open into the median stem of the vesicle a short distance behind the lateral branches. These ducts pass laterad and anteriad and near the middle of the body each bifurcates to form an anterior and a posterior branch. The pattern is the same in each quadrant of the body and consists of three sets of tubules, each receiving the capillaries from three flame cells as shown in Figure 4. The flame-cell formula of the mature cercaria is $2(3 + 3 + 3) + (3 + 3 + 3)$. In earlier literature, the idea is expressed that flame cells and capillaries divide to form the pattern of the mature cercaria, but there appears to be no critical evidence to support this idea and I have never seen a dividing flame cell or one in which there were two nuclei. It seems unlikely that functional flame cells divide and I am inclined to agree with Willey (1936) that they arise from undifferentiated cells in the parenchyma. It is probable that physiological conditions, perhaps the accumulation of excretory wastes, induce the formation of new flame cells from undifferentiated mesenchyme cells.

As mentioned above, the body of the cercaria is so filled with gland cells that other structures are obscured and the organs of later stages are relatively undeveloped. A cluster of cells anterior to the acetabulum probably represents the rudiment of the copulatory organs and genital pore.

The cercariae may encyst in their sporocysts if left in sea-water; they will penetrate and encyst in excised parapodia of *Neanthes virens,* in *Littorina saxatilis, L. littorea* and *L. obtusata,* and in the small polyclad turbellarian, *Euplana gracilis.* In the turbellarian, the cysts were extruded after a few days. Newly formed cysts are spherical to oval, 0.1–0.15 mm. in diameter. The cyst wall is 0.002–0.0025 mm. thick, flexible and very tough. The cysts are not encapsulated by snail tissue and readily fall free when the snail is dissected. While the cysts are spherical in the host tissue, when released in sea-water they become ovate, with the body of the metacercaria bent and the ends of the worm at the more pointed end of the cyst. On each side of the mouth of the larva, there is a row of 12–15 spines, larger than those regularly present in the cuticula.

Table I

	Cercaria roscovita	Cercaria parvicaudata
Host	*Littorina saxatilis nigrolineata*	*Littorina littorea, L. saxatilis, L. obtusata*
Sporocyst	Lemon colored, 0.7–1.5 mm. long, 0.25–0.45 mm. wide	Orange colored, 0.3–0.9 mm. long, 0.15–0.35 mm. wide
Cercaria	0.15–0.3 mm. long, 0.06–0.12 mm. wide	0.14–0.36 mm. long, 0.06–0.12 mm. wide
Tail	Subterminal, shorter than body, 0.08–0.3 mm. long	Same, 0.06–0.3 mm. long
Acetabulum	Postcentral, 0.034–0.05 mm. in diameter	Same
Stylet	Simple, 0.016–0.018 mm. long, 0.002–0.003 mm. wide	Simple, 0.015 mm. long, 0.0032 mm. wide
Cuticula	Sharp pointed spines	Same
Oral sucker	0.042–0.05 mm. in diameter	0.035–0.06 mm. in diameter
Pharynx	Near oral sucker, 0.014–0.018 mm. in diameter	Same, 0.012–0.014 mm. in diameter
Esophagus	Ends midway between suckers	Same
Penetration glands	Several, preacetabular, stain rose pink with neutral red	6 pairs, preacetabular, same staining reaction
Cystogenous glands	Numerous, secretion extruded under pressure	Same
Refractive globules	Very numerous in intercellular spaces	Same
Excretory vesicle	Y-shaped, collecting ducts enter stem of Y	Same
Flame-cell formula	2(3 + 3 + 3) + (3 + 3 + 3)	Same

DISCUSSION

The morphology of *C. parvicaudata* is strikingly similar to that of *C. roscovita* described by Stunkard (1932) from *Littorina saxatilis nigrolineata* taken at Roscoff, France. The two species are unique in that the collecting ducts of the excretory system open into the stem rather than into the lateral branches of the excretory vesicle. Comparison of the two species is facilitated by Table I.

Except for the difference in color of the sporocysts, the two species are almost identical. Differences in size may result from conditions under which the observations were made, *e. g.*, the amount of pressure to which the specimens were subjected at the time of measurement. The general structure of the two species, and especially the details of the excretory system, suggest that they are not only closely related but that they are probably members of the family Plagiorchiidae. Since members of this family are rare or unknown in marine fishes, and occur most frequently in birds and mammals, it is probable that some bird or mammal which frequents the sea-shore is the final host of *Cercaria parvicaudata.*

LITERATURE CITED

STEPHENSON, WILLIAM, 1947. Physiological and histochemical observations on the adult liver fluke, *Fasciola hepatica* L. IV. The excretory system. *Parasitology*, **38**: 140–144.

STUNKARD, H. W., 1932. Some larval trematodes from the coast in the region of Roscoff, Finistère. *Parasitology*, **24**: 321–343.

STUNKARD, H. W. AND R. M. CABLE, 1932. The life history of *Parorchis avitus* (Linton), a trematode from the cloaca of the gull. *Biol. Bull.*, **62**: 328–338.

STUNKARD, H. W. AND C. R. SHAW, 1931. The effect of dilution of sea water on the activity and longevity of certain marine cercariae, with descriptions of two new species. *Biol. Bull.*, **61**: 242–271.

WILLEY, C. H., 1936. Cytology and development of flame cells in the redial generation of a trematode. *Anat. Rec.*, **67** (suppl.) : 85.

Vol. 99, No. 2 October, 1950

THE

BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

OBSERVATIONS ON POLARITY IN THE SLIME MOLD DICTYOSTELIUM DISCOIDEUM

JOHN TYLER BONNER[1]

Princeton University

In previous work done on the amoeboid slime mold, *Dictyostelium discoideum,* there was good evidence that a chemical substance called acrasin is involved in all its morphogenetic stages. The substance was first demonstrated as responsible for the bringing together of the myxamoebae by chemotaxis in the aggregation stage (Bonner, 1947). Then it was shown that the same substance was continually emitted, in varying amounts depending on the stage of development and the part of the cell mass or pseudoplasmodium, until the final, mature spore-bearing fruiting body was formed (Bonner, 1949). During these later migration and final culmination stages (Raper, 1935, 1940a; Bonner, 1944, for details of the life history) it was shown that there was a high correlation between the amount of acrasin produced by a region and the differentiation of that region into spore or stalk cells (Bonner and Slifkin, 1949).

There are, in the morphogenesis of this slime mold, certain manifestations that can be grouped under the general term polarity, a word which implies a directional quality, a headness and tailness, a symmetry to the developing organism. The question before us (and this paper by no means answers it, but only clarifies the issue to some extent) is what precisely is the relation between polarity and the acrasin mechanism. The mere fact that it is always the anterior end of the migrating pseudoplasmodium that becomes the high region of acrasin emission shows that the two processes are interconnected, but we know little more than this. Polarity is so fundamental and yet so mysterious an aspect of development that any description of some of its activities, which is all I offer here, might ultimately help in guiding us to the real answers.

OBSERVATIONS AND EXPERIMENTS

Vegetative myxamoebae. Polarity in any amoeba presents a special problem, for in most organisms polarity refers to a fixity of direction which is expressed by the fixity of the parts, and in an amoeba there is little fixity of parts. An amoeba may crawl in one direction for a great period of time, but in doing so practically every particle of its substance will pass a moment, however fleeting, at its anterior end, or at any other point. It is true that the nucleus will remain somewhere in

---

[1] This work was carried out with the help of a grant from the American Cancer Society.

the very general vicinity of the center and also there are occasional evidences of a somewhat more permanent foot or posterior region, but even these phenomena show great variation from moment to moment. And some years ago Jennings (1904) showed, by watching small particles that adhere to the outside surface of an ordinary amoeba, that the surface membrane itself moves in relation to the whole amoeba, by a sort of rolling action.

At any one instant vegetative myxamoebae have a polar appearance, for although their contour is irregular and amorphous, one section of their border (approximately $\frac{1}{4}$ to $\frac{1}{2}$ of the circumference) is hyaline, while the rest is granular. This is well illustrated in some drawings by Raper (1941). But, just as we have seen in the case of the true amoebae, this polarity is ephemeral.

Aggregating myxamoebae. The myxamoebae themselves, when they commence aggregating, are more elongated than during the vegetative stage, stretching in length to 5 or 6 times their diameter (Bonner, 1947, for photographs of aggregating myxamoebae). But nevertheless, the problems which were mentioned for the vegetative myxamoebae concerning the constant mixing and reshuffling of the internal parts, apply equally well for the elongate aggregating myxamoebae. There is a curious cap on the nucleus which can be seen in stained preparations and with the phase contrast microscope, but even it is not consistently oriented in respect to the aggregation center but is pointed about 50 per cent of the time towards the center and 50 per cent away.

The nearest phenomenon to fixity of parts that is found in aggregating myxamoebae is that the nucleus usually lies in the anterior half of the cell. This is clearly seen in living myxamoebae with an oil immersion phase contrast microscope.[2] The nucleus does, of course, jostle about severely during movement, but its position is most often in the second quarter of the cell from the anterior end. The anterior-most quarter is hyaline and lacks any visible inclusions whatsoever.

Since the aggregating myxamoebae are presumably oriented in their movement by a diffusion gradient of acrasin, it seemed of interest to see what effect suddenly reversing the acrasin gradient might have on them. This was first done in repeated experiments on isolated aggregating myxamoebae and on myxamoebae in thin streams. The center towards which they were moving was simply removed and placed directly behind them. These were done in under-water preparations, using standard solution (Bonner, 1947). Invariably one of two things happened. Occasionally the myxamoebae balled up and after considerable kneading of the surface of the sphere it would send out a pseudopod in the direction of the center at its new location. More frequently the myxamoeba would make a "U" turn without ever losing its elongate shape (see Fig. 1, A).

In another type of experiment a section of a stream of incoming myxamoebae was removed, and reversed. The section, usually about 200 μ long, was cut with a glass needle and turned 180°. If the piece was kept separate from the remaining stream it first contracted and then the individual myxamoebae separated from the piece and moved independently towards the new center (Fig. 1, B). No difference in behavior could be detected between cases where the section was cut and reversed, or merely cut. If the reversed piece was touched to the remaining stump of the stream connected to the center, then they adhered fast to one another. Almost immediately after the operation the reversed section became short and thick and it could be seen that this was because each myxamoeba had become rounded. This rounded appearance of the myxamoebae first reverted to the normal elongated form at the central end of the reversed section as though the myxamoebae had been pulled into their normal elongate shape. In each attempt this passage from the rounded to the elongated form progressed distally until a stream normal in appearance was achieved (Fig. 1, C).

---

[2] The technique used here was the "thin film of water technique" given in detail by Bonner (1947). The only modification was that the coverslip containing the myxamoebae was placed over a van Tieghem cell filled with light Parke-Davis mineral oil. This greatly enhanced the optical conditions for the phase microscope. Bausch and Lomb phase contrast equipment was used: a 97 × objective, and a long working distance condenser.

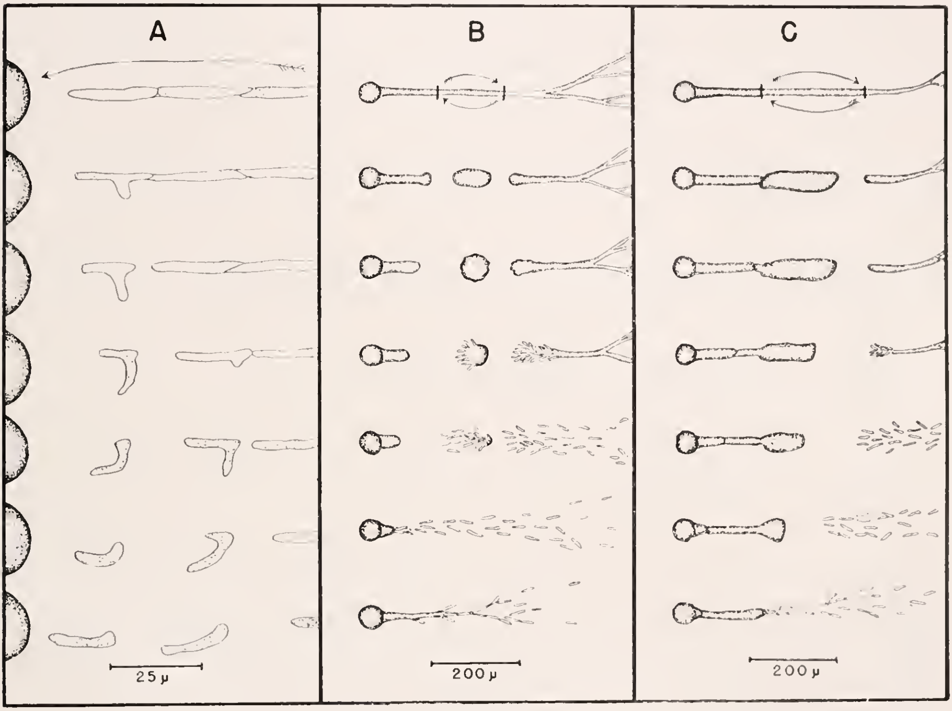


FIGURE 1. Diagram illustrating the experimental reversal of an acrasin gradient during aggregation. A. In the top diagram a center has been removed from in front of the chain of myxamoebae and placed behind. The succeeding drawings show the resulting changes at intervals of 2 min. B. A section of the stream has been reversed (top) and the resulting changes are shown at 4 min. intervals. C. The same as B except that the reversed piece was attached to the proximal stump of the stream.

One last curious phenomenon concerning aggregation should be mentioned here and that is the observation of Arndt on aggregation centers that form whirlpools (Arndt, 1937; Raper, 1941). Instead of the amoebae coming directly to a point, they come in at an angle (exactly the way water enters a whirlpool) and the cell mass is not solid, but ring-shaped with a hollow center. Using the under-water preparations (Bonner, 1947) whirlpool centers often appear and if one watches the ring one can see that the cells follow each other round and round, the ring constantly decreasing in diameter, until the hole in the center disappears; forming finally a solid cell mass. But the important point here is that the cells in the ring move in a polar fashion, even though there is no dominant point, but only a continuous ring.

Migrating pseudoplasmodia. In its external form the migrating pseudoplasmodium is obviously and clearly polar. The anterior end is radially symmetrical and tapers down to a characteristic "bullet-nose," while the posterior end is broad and bilaterally symmetrical, tapering down to a small flat tail. Furthermore the motion shows a fixed polarity and a slime track is deposited posteriorly, that is, it comes off the posterior end.

Previously (Bonner, 1944) serial sections were made of migrating pseudoplasmodia and it was noted that ordinarily there was no marked orientation of the myxamoebae within the pseudoplasmodium except in the anterior portion of late migrating pseudoplasmodia. There the long axis of the cells was at right angles to the long axis of the whole pseudoplasmodium. Upon careful re-examination of the slides it can be seen that the posterior cells occasionally show slight orientation in the antero-posterior direction, most frequently in the cells near the slime sheath. Perpendicular orientation of the anterior cells was also observed in the pre-stalk cells of the tip of the culminating pseudoplasmodium.

A series of studies were made in which the pseudoplasmodia were made to crawl on the underneath side of coverslips, and the cells were observed with the high powers of the microscope (43 × and 97 × objectives). Invariably all the cells that could be seen were actively involved in amoeboid motion continually giving off pseudopodia. With these preparations it was impossible to see the perpendicularly oriented cells in the anterior end, but it was found that if a culminating pseudoplasmodium was placed in a small drop of mineral oil between a slide and a coverslip and the preparation was observed with the phase contrast microscope (43 × objective) the outlines of the cells could be seen.[3] Motion pictures of this were taken with a 16 mm. movie camera at 500 × normal speed, and these showed an even, smooth forward movement towards the tip of all the cells, despite their perpendicular orientation relative to their axis of motion.

It is difficult to reconcile the fact that the long axes of the constituents cells do not always lie in the same direction of the long axis of the whole migrating pseudoplasmodium. Another problem is that since the cells of migrating pseudoplasmodia continue to be pseudopodial, presumably they too (as did the isolated myxamoebae) must have their parts in a continual flux. The puzzling question here is how it is that with each cell in constant turmoil and showing little or no orientation with respect to its movement, that the whole migrating pseudoplasmodium shows polarity of shape and of movement. In an effort to analyze this problem further, experiments attempting to reverse sections of migrating pseudoplasmodia were made.

The first experiments involving reversing sections of migrating pseudoplasmodia were done by Raper (1940b). He found that if he cut off a posterior section and replaced it after reversing it 180°, then invariably the pieces crawled away from one another; never fusing and always retaining their original direction of movement. This experiment is easy to perform and I have obtained the same results with many tries.

---

[3] A different species *Polysphondylium violaceum* was used here as they were somewhat easier to handle for this purpose. No difference in respect to the orientation could be observed between it and *Dictyostelium discoideum.*

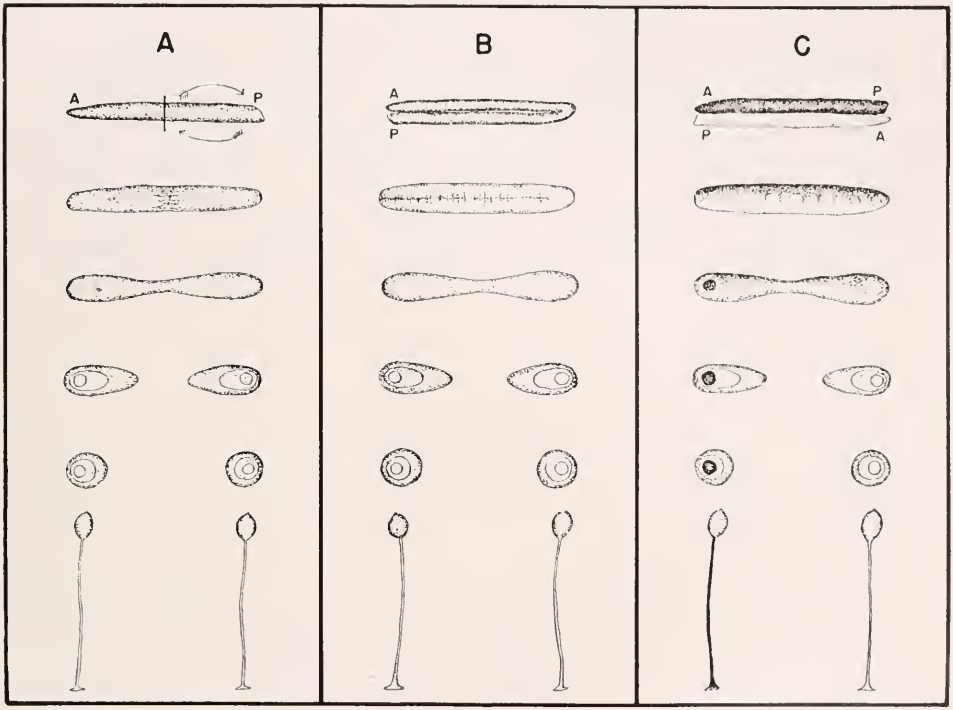


FIGURE 2. Diagram showing the experimental reversal of parts of the migrating pseudoplasmodium. A. The posterior half of a migrating pseudoplasmodium has been reversed and fused carefully onto the anterior half. The changes resulting are given at 15 min. intervals except for the final fruiting bodies drawn at the bottom which have appeared after an interval of 10 hrs. (For convenience the mature fruiting bodies are represented disproportionately small in this figure.) B. A migrating pseudoplasmodium is folded onto itself and the resulting changes are recorded as described for A. C. Two migrating pseudoplasmodia oriented in opposite directions and the upper one stained with nile blue sulphate are carefully fused and the changes are recorded as described for A. Note that the blue and colorless cells intermingle except at the anterior tips.

The point in question here is whether or not, provided the conditions set up in the experiment are just right, a section can be made to reverse if it is under the influence of a high source of acrasin in the close vicinity. One of the possible difficulties encountered in the type of experiment Raper used might be that the slime sheath crowded or herded the cells together, thereby physically preventing them from responding to acrasin. In order to avoid this after a section was reversed, it was gently stirred with an extremely fine glass needle so as to rupture the slime sheath, and carefully fused in this way to the unreversed section. Again the results were the same, for despite this disruption of the sheath the original polarity of each piece was retained bearing no relation to the overall acrasin emission gradients (Fig. 2, A).

A number of variations of these experiments were undertaken and in each case the object was to cause a reversal of the polarity of a part of the pseudoplasmodium.

In one variation, pseudoplasmodia were folded back so that the two halves touched and the anterior and posterior ends were just opposite each other. The halves were then fused together by passing a fine glass needle back and forth through them. This folded pseudoplasmodium would soon divide into two, forming one center at the "anterior-posterior" end, and another at the "middle" end. These centers were of approximately equal size indicating that the cells retained their original direction of motion, rather than being influenced by the presumably dominant tip region (Fig. 2, B).

In another group of experiments, whole migrating pseudoplasmodia were placed along side each other so that the anterior end of one would lie along side the posterior end of the other. Again they were fused with a needle and again two pseudoplasmodia about equal in size formed at each end of the mass of cells. In some cases the anterior $\frac{1}{10}$ of one of the pseudoplasmodia was removed, but the result of the experiment was the same.[4]

These same experiments were repeated but one of the pseudoplasmodia was stained with nile blue sulphate. Then, after fusion, it was possible to follow the cells of the two pseudoplasmodia. Invariably the two new centers contained both blue and colorless cells, although the center which arose at the site of the anterior end of the blue pseudoplasmodium was predominantly blue and the converse was true of the other center (Fig. 2, C). So we see that although the original polarities of the two pseudoplasmodia were maintained there was considerable mixing of the cells. It was, of course, impossible to ascertain to what extent this was due to the reversing of cells by literally turning them about with the needle, but conceivably this could completely account for the phenomenon.

One final experiment on the migrating pseudoplasmodia should be mentioned, in which pseudoplasmodia were coiled in a ring so that their anterior ends just touched their posterior ends. The two ends were then fused with a needle. After a period of 15 minutes or so, a separation line would appear at its original place and the anterior end would move off in another direction. This would occur even in cases where there was known to be an even acrasin emission the length of the pseudoplasmodium, and in cases where the whole ring was gently stirred with a needle. Apparently the difference between the anterior and posterior ends is sufficient so that their individuality is retained.

Shaking experiments. Using the technique of obtaining myxamoebae for under water preparations (Bonner, 1947), washed myxamoebae in standard solution were placed in 125 cc. Erlenmeyer flasks. The flasks were set on a shaking machine which gently rocked them back and forth. Even though the myxamoebae were at their vegetative stage, after about 5 to 10 minutes of shaking they clumped into spherical balls of cells. With continuous shaking for 12 hours these balls of cells became quite polar in appearance, for one end (presumably anterior) was relatively pointed, the other end (posterior) exuded slime sheath material (Fig. 3). It is quite remarkable that the over-all polarity becomes evident in a spherical ball of cells under constant agitation. These polar masses were placed among random myxamoebae which oriented towards the mass, indicating that the mass was actively emitting acrasin.

---

[4] It should be mentioned that in all these experiments, if the disrupting with the needle is sufficiently great, many small centers will arise and therefore care was taken to apply the needle gently.

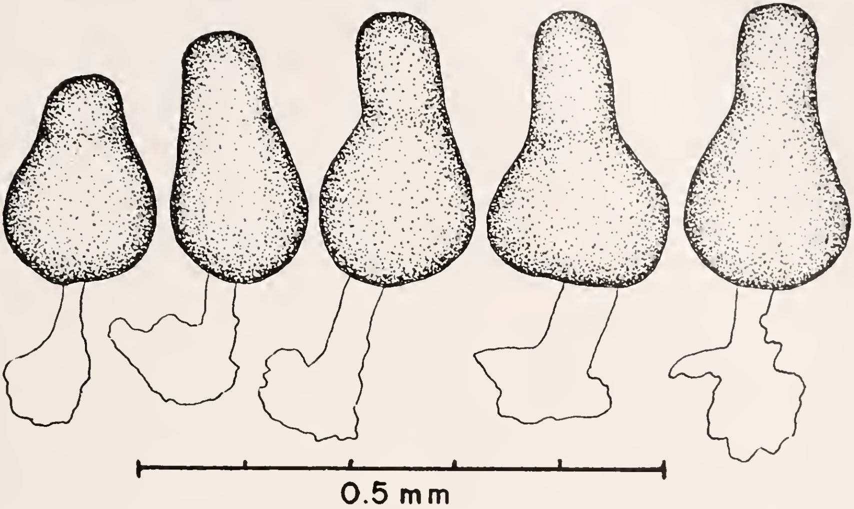


FIGURE 3. Camera lucida drawings of a number of the regular cell masses appearing after 12 hrs. of shaking. Note the slime sheath material issuing from one end. These drawings are of cell masses from one flask and are an example of the most regular forms obtained.

DISCUSSION

One fact that clearly emerges from the above observations is that the over-all, total morphological polarity shows a consistent relation with the polarity of movement, but the smaller constituent parts show little or no relation. In aggregation the external contour of the myxamoebae reflects its polar movement, and also in migration the direction of movement is reflected by the shape of the whole pseudoplasmodium. But in aggregation the parts of the myxamoebae show no such relation (with the possible exception of the position of the nucleus) and in migration, not only the internal parts of the myxamoebae, but also the myxamoebae themselves do not orient with respect to the polarity of the migrating pseudoplasmodium.

It is clear that however the polarity arises in the migrating pseudoplasmodium, it is not necessarily dependent on the polarity acquired during aggregation, for in the shaking experiments it was shown that the polarity could arise in a ball of cells thrown together quickly. The experiments in which the migrating pseudoplasmodia were placed in a ring show that not only is the direction of the movement important, but that there are discreet (although unknown) differences between the anterior and the posterior end, for these will not fuse but separate from one another after artificial fusion.

In aggregation the gradient of acrasin is capable of governing the polarity, for the myxamoebae invariably orient towards a high concentration of acrasin (Bonner, 1947). However, this is probably not the only factor involved for the adhesiveness of the myxamoebae played a part in the experiment where a section of reversed aggregation stream was attached to another stream and literally pulled into the center (Fig. 1, C).

The best evidence that an external acrasin gradient is not necessarily required for orienting the aggregating myxamoebae comes from the whirlpool configuration, for in that case there is a line of myxamoebae moving in a polar fashion, yet presumably they are all in the same external concentration of acrasin. Even if there were a high point of acrasin production at some region on the ring, then the argument that the acrasin gradient is not orienting the myxamoebae would still hold: for some of the myxamoebae in the ring must be moving away from the region of high acrasin concentration.

In the migrating pseudoplasmodium there is also abundant evidence that an over-all external acrasin gradient does not necessarily govern the polar movement. In the first place, even though the young migrating pseudoplasmodia move about with over-all polarity, they do not possess a gradient of acrasin emission but emit the acrasin equally in all parts (Bonner and Slifkin, 1949). Also, in the experiments in which portions of the pseudoplasmodia were reversed, in no case did the movement of the sections reverse and go towards a high acrasin producing tip (in older pseudoplasmodia the tip produces more acrasin than the remaining portion, Bonner, 1949).

We have then a good demonstration that an over-all acrasin gradient does not account for polar movement or polar shape. There is, unfortunately, no way as yet of determining the distribution of acrasin inside the cell mass, for it is not at all certain that the over-all emission of acrasin reflects its internal distribution at all.

SUMMARY

Experiments designed to give some insight into the nature of polarity were performed on the amoeboid slime mold *Dictyostelium discoideum*. The separate myxamoebae which stream together to form aggregations of myxamoebae show an external polarity and the resulting cell mass has a clear over-all antero-posteriority. In both cases it was shown that the internal parts do not reflect the external polarity. It is known from previous experiments that the aggregating myxamoebae orient in a concentration gradient of a chemical substance tentatively called acrasin, and that acrasin is found in later stages of development. By reversing the gradient of acrasin, separate myxamoebae did not back up but re-oriented towards the point of high concentration. However, it was shown by various experiments that the external acrasin gradient cannot always determine the direction of the polarity, for in the cell masses the polar movement occurs when there is no external gradient of acrasin, or in some cases away from the region of high acrasin emission. It was also shown that polarity can arise in spherical cell masses undergoing gentle shaking in liquid media.

LITERATURE CITED

Arndt, A., 1937. Untersuchungen über Dictyostelium mucoroides Brefeld. *Roux' Arch. Entwmech.*, **136**: 681–747.

Bonner, J. T., 1944. A descriptive study of the development of the slime mold *Dictyostelium discoideum*. *Amer. Jour. Bot.*, **31**: 175–182.

Bonner, J. T., 1947. Evidence for the formation of cell aggregates by chemotaxis in the development of the slime mold *Dictyostelium discoideum*. *Jour. Exp. Zool.*, **106**: 1–26.

Bonner, J. T., 1949. The demonstration of acrasin in the later stages of development of the slime mold *Dictyostelium discoideum*. *Jour. Exper. Zool.*, **110**: 259–272.

Bonner, J. T. and M. K. Slifkin, 1949. A study of the control of differentiation: the proportions of stalk and spore cells in the slime mold *Dictyostelium discoideum*. *Amer. Jour. Bot.*, **36**: 727–734.

Jennings, H. S., 1904. Contribution to the study of the behavior of lower organisms. Publ. Carnegie Institution, Washington.

Raper, K. B., 1935. *Dictyostelium discoideum*, a new species of slime mold from decaying forest leaves. *Jour. Agric. Res.*, **50**: 135–147.

Raper, K. B., 1940a. The communal nature of the fruiting process of the Acrasieae. *Amer. Jour. Bot.*, **27**: 436–448.

Raper, K. B., 1940b. Pseudoplasmodium formation and organization in *Dictyostelium discoideum*. *Jour. Elisha Mitchell Sci. Soc.*, **56**: 241–282.

Raper, K. B., 1941. Developmental patterns in simple slime molds. *Growth* (*Third Growth Symposium*), **5**: 41–76.

ALKALINE PHOSPHATASE ACTIVITY IN KIDNEYS OF GLOMERULAR AND AGLOMERULAR MARINE TELEOSTS

MARIE J. BROWNE, MARJORIE W. PITTS AND ROBERT F. PITTS[1]

The Department of Physiology, Syracuse University College of Medicine, the Marine Biological Laboratory, Woods Hole, Mass., and the Mt. Desert Island Biological Laboratory, Salisbury Cove, Maine

It is generally accepted that glucose undergoes phosphorylation in the process of absorption from the glomerular filtrate (Shannon, 1942). Formation within the tubular cells of a hexose phosphate ester is presumed to create the diffusion gradient which permits a continuous flow of glucose across the lumenal membrane (Kalckar, 1941). Subsequently the ester is hydrolyzed and the glucose discharged into the peritubular blood stream. Presumably phosphorylation is effected at the expense of the labile phosphate bond energy of adenosine triphosphate. In favor of this concept is the presence within the cells of the proximal segments of the renal tubules of rich stores of ATP, hexokinase, and alkaline phosphatase (Kalckar, 1937), the latter two enzymes being necessary, respectively, for phosphorylation of glucose and hydrolysis of the ester. Furthermore, glucose is known to be absorbed solely by cells of the proximal segment (Walker *et al.*, 1941; Walker and Hudson, 1937) which alone contain the enzyme phosphatase in its characteristic location in the brush border (Gomori, 1941). In addition, the absorptive mechanism is an energy consuming one of limited transfer capacity (Shannon and Fischer, 1938) which can be partially or completely blocked by such enzyme inhibitors as cyanide (Bayliss and Lundsgaard, 1928), phlorizin (Lundsgaard, 1933) and mercury (Weston *et al.*, 1949).

Were alkaline phosphatase to be concerned only with the hydrolysis of the hexose ester formed within the tubular cells in the reactions outlined above, it should be absent in those forms which possess no glomeruli, filter no glucose and hence need and presumably have no glucose absorptive mechanism. According to Wilmer (1944) the renal tubules of the toadfish, an aglomerular marine teleost, are devoid of alkaline phosphatase, whereas those of fresh water glomerular teleosts exhibit an abundance of the enzyme. Kalckar (1941) in a similar vein states "aglomerular kidneys (toadfish) contain much less phosphatase than glomerular kidneys from closely related species." If these observations are correct, they constitute strong evidence that renal alkaline phosphatase, at least in the fish kidney, is largely concerned with the absorption of glucose, for the urines of glomerular and aglomerular forms do not differ appreciably in composition (Marshall, 1930).

To test the above hypothesis, the distribution of alkaline phosphatase has been studied in a representative series of marine fish. Because it has been found that the enzyme is present in the brush border of the "proximal segment" of both aglomerular and glomerular forms, it must be concerned with renal tubular functions in addition to, or other than, the absorption of glucose from the glomerular filtrate.

---

[1] Aided by a grant from the United States Public Health Service.

METHODS

At least three specimens of each of the following fish were caught in traps or seines by the collecting crew of the Marine Biological Laboratory or by commercial dredgers off Menemsha Bight: sea robin (*Prionotus evolans strigatus*), puffer (*Tetraodon maculatus*), eel (*Anguilla rostrata*), dogfish (*Mustelus canis*), skate (*Raja erinacea*), toadfish (*Opsanus tau*), goosefish (*Lophius piscatorius*), and pipefish (*Syngnathus fuscus*). The latter three are aglomerular fish (Marshall, 1934). In addition, specimens of the daddy sculpin (*Myoxycelphalus scorpius*), the long horn sculpin (*Myoxycephalus octodecimspinosus*) and the flounder (*Pseudopleuronectes americanus*) were caught by hook and line at the Mt. Desert Island Biological Laboratory. The daddy sculpin, when full grown, is essentially aglomerular (Grafflin, 1933). All were killed by a blow on the head; the kidneys were promptly removed, and small blocks of tissue were fixed in 95 per cent alcohol for 14 to 20 hours according to the method of Gomori (1939). Further handling, including dehydration and embedding of the tissue in paraffin, was performed as outlined in this method. Sections were cut 6 microns in thickness and those from three to six different species were mounted on a single slide to compare enzymatic activity under identical conditions of treatment. The modified method of Krugelis (1946) was employed to demonstrate the presence of alkaline phosphatase and the slides were subsequently counterstained with light green. Incubation in alkaline glycerophosphate was carried out at 37° C. for ½ to 21 hours. Two types of controls were run: (a) preformed calcium deposits were visualized by cobalt exchange and precipitation by potassium sulfide; (b) the entire incubation and development procedure was carried out, except that glycerophosphate was omitted from the reaction mixture.

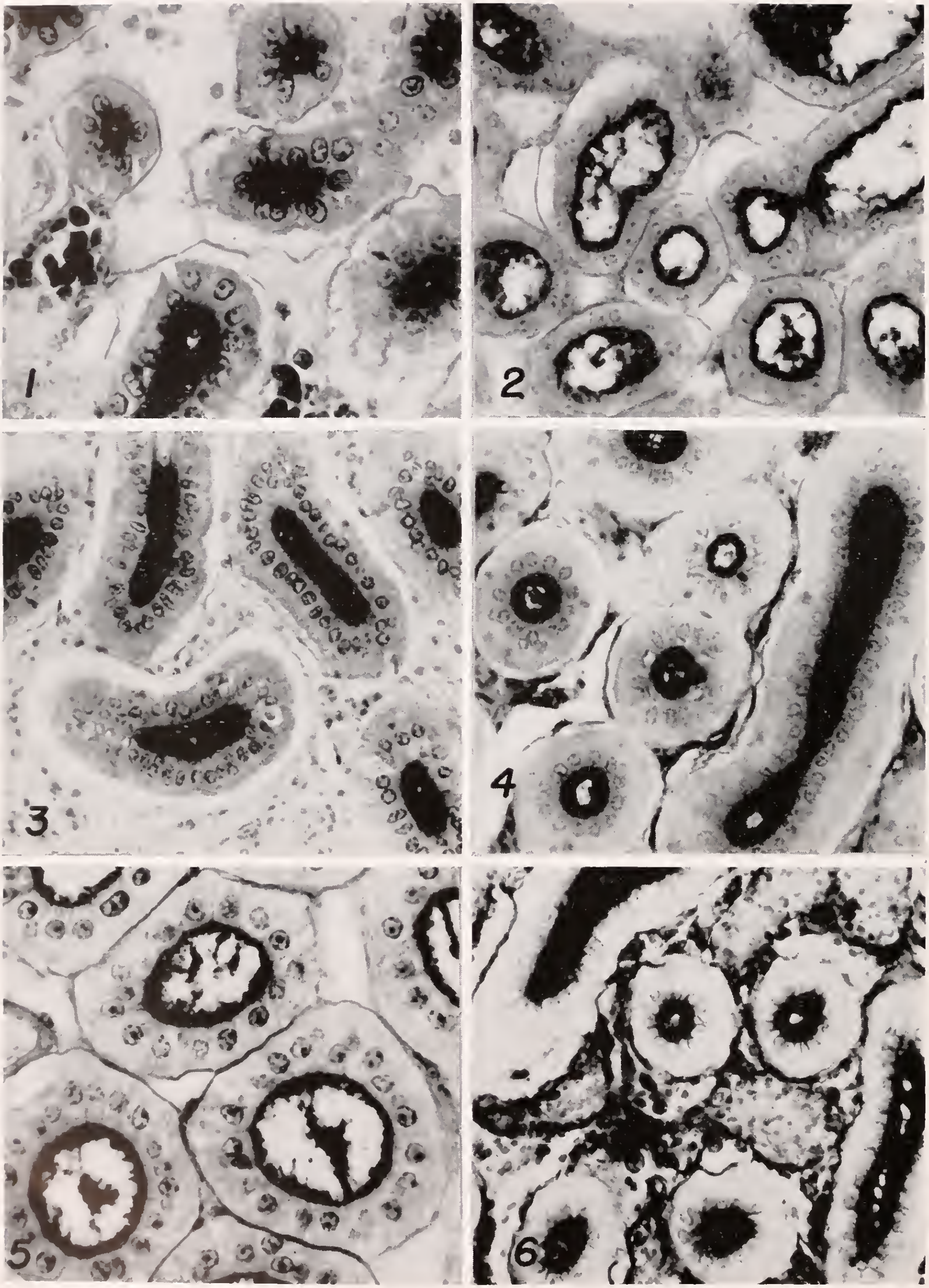
RESULTS

Alkaline phosphatase activity could be demonstrated readily in its characteristic location in the brush border of the "proximal" tubular segments of ten of the eleven species of marine fish examined, including all of the aglomerular forms. The only fish in which no activity could be demonstrated in this location, in any one of three specimens, was the eel. In view of the fact that fixation in all blocks from all of these specimens was, for some unknown reason, very poor, we attach no significance to this finding. Plate I, Figures 1 through 6, illustrates in photomicrographs the distribution of the enzyme in six fish: two glomerular teleosts (sculpin and sea robin), two aglomerular teleosts (toadfish and pipefish), one nearly aglomerular teleost (daddy sculpin) [2] and one glomerular selachian (skate). It is apparent that activity is demonstrable in the brush border, in the nuclei of the tubular cells, in the interstitial tissue (so called pseudolymphatic tissue, Marshall and Smith, 1930), in the glomerular capillaries and in some parts of the peritubular network of vessels. Little or none is demonstrable in the cytoplasm of the tubular cells. Preformed deposits of calcium of any magnitude could be demonstrated only in the glomeruli of the eel and in the connective tissue of the renal capsule of the pipefish. When all steps of the procedure were carried out in the usual fashion, except that

---

[2] This daddy sculpin weighed 570 gms. According to Grafflin (1933), glomerular degeneration should be essentially complete.

PLATE I



glycerophosphate was omitted from the incubation mixture, no precipitate was observed other than that of preformed calcium in the instances noted above.

DISCUSSION

We doubt that quantitative comparisons of enzyme activity (based on incubation time necessary to obtain a given density of precipitate) have, in this material, much cytochemical significance. Although the reaction is without caprice when applied on different occasions to sections from a given block of tissue, sections from different specimens of a given species vary considerably in reactivity. Even sections from different blocks of a given specimen vary somewhat, indicating that minor differences in fixation may account for variability. However, from the limited numbers of specimens in our series, it would appear that the sea robin, skate, pipefish, and toadfish give the most intense brush border reaction. Nevertheless, in one specimen of toadfish it was necessary to incubate for 21 hours at 37° C. to demonstrate enzyme activity adequately. We infer that the failure of Wilmer (1944) and Kalckar (1941) to observe the enzyme in the brush border of the toadfish may be related to inadequacy of fixation, and/or insufficient time of incubation.

Although our results do not in any sense negate the possibility that the alkaline phosphatase of the brush border of the proximal segment of glomerular kidneys functions in the absorption of glucose, they do suggest strongly that the enzyme is concerned in other tubular functions shared by aglomerular and glomerular forms.

SUMMARY

The kidneys of 35 specimens of 11 marine fish have been studied by histochemical methods for the presence and distribution of alkaline phosphatase. All fish examined, with the exception of the eel, exhibited alkaline phosphatase in the brush border of the "proximal" segment. Since the ten species which exhibited the characteristic location of the enzyme included three aglomerular teleosts (toadfish, pipefish, and goosefish), one equivocal aglomerular teleost (daddy sculpin), four glomerular teleosts (sea robin, puffer, flounder, and long horn sculpin), and two

---

PLATE I

Photomicrographs of sections of the renal tubules of a series of marine fish. All sections 6 micra in thickness; all photomicrographs 650 ×. Alkaline phosphatase activity was demonstrated by precipitation of black cobaltous sulfide and the sections were subsequently counterstained with light green. Variations in time of incubation (noted below) were used to clarify histological structure and localize activity rather than to quantify the amount of enzyme present. Evidence of alkaline phosphatase activity is seen in the brush border and nuclei of the tubular cells and in the interstitial tissue, but only to a negligible degree in the cytoplasm of the tubular cells. Figures 1 and 2 are from aglomerular fish; Figure 3 is from an essentially aglomerular fish; Figures 4–6 are from glomerular fish.

FIGURE 1. Renal tubules of the toadfish. Incubation time 4 hours.
FIGURE 2. Renal tubules of the pipefish. Incubation time 6 hours.
FIGURE 3. Renal tubules of the daddy sculpin. Incubation time 21 hours.
FIGURE 4. Renal tubules of the long horn sculpin. Incubation time 2 hours.
FIGURE 5. Renal tubules of the skate. Incubation time 4 hours.
FIGURE 6. Renal tubules of the sea robin. Incubation time 1 hour.

glomerular selachians (skate and dogfish), it is concluded that the enzyme of the brush border must be concerned in tubular processes shared by forms with and without glomeruli. It cannot, therefore, function solely in the absorption of glucose from the glomerular filtrate.

The authors are indebted to Miss Stella Zimmer for the preparation of the photomicrographs.

LITERATURE CITED

Bayliss, L. E. and E. Lundsgaard, 1928. The action of cyanide on the isolated mammalian kidney. *J. Physiol.*, **74**: 279.

Gomori, G., 1939. Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. Exp. Biol. and Med.*, **42**: 23.

Gomori, G., 1941. The distribution of phosphatase in normal organs and tissues. *J. Cell. and Comp. Physiol.*, **17**: 71.

Grafflin, A. L., 1933. Glomerular degeneration in the kidney of the daddy sculpin. *Anat. Record*, **57**: 59.

Kalckar, H., 1937. The significance of phosphorylation in kidney tissue. *Skand. Arch. f. Physiol.*, **77**: 46.

Kalckar, H., 1941. The nature of energetic coupling in biological synthesis. *Chem. Rev.*, **28**: 71.

Krugelis, Edith J., 1946. Distribution of intracellular alkaline phosphatase. *Biol. Bull.*, **90**: 220.

Lundsgaard, E., 1933. Hemmung von Esterifizierungsvorgängen als Ursache der Phlorrhizinwirkung. *Biochem. Zeitschr.*, **264**: 209.

Marshall, E. K., Jr., 1930. A comparison of the function of the glomerular and aglomerular kidney. *Amer. J. Physiol.*, **94**: 1.

Marshall, E. K., Jr., 1934. The comparative physiology of the kidney in relation to theories of renal secretion. *Physiol. Rev.*, **14**: 133.

Marshall, E. K., Jr., and H. W. Smith, 1930. The glomerular development of the vertebrate kidney in relation to habitat. *Biol. Bull.*, **59**: 135.

Shannon, J. A., 1942. Kidney. *Ann. Rev. Physiol.*, **4**: 297.

Shannon, J. A. and S. Fischer, 1938. The renal tubular reabsorption of glucose in the normal dog. *Amer. J. Physiol.*, **122**: 765.

Walker, A. M., P. A. Bott, J. Oliver and M. C. MacDowell, 1941. The collection and analysis of fluid from single nephrons of the mammalian kidney. *Am. J. Physiol.*, **134**: 580.

Walker, A. M. and C. L. Hudson, 1937. The reabsorption of glucose from the renal tubule of the amphibian kidney and the action of phloridzin upon it. *Am. J. Physiol.*, **118**: 130.

Weston, R. E., J. Grossman, I. S. Edelman, D. J. W. Escher and L. Hellman, 1949. Renal tubular action of diuretics; effects of mercurial diuresis on glucose absorption. *Federation Proc.*, **8**: 164.

Wilmer, H. A., 1944. Renal phosphatase; a correlation between functional activity of the renal tubule and its alkaline phosphatase content. *Arch. Pathology*, **37**: 227.

PHOTOSYNTHESIS AND PHOTOREDUCTION BY THE BLUE GREEN ALGA, SYNECHOCOCCUS ELONGATUS, NÄG.

ALBERT FRENKEL, HANS GAFFRON AND EDWIN H. BATTLEY

Department of Botany, University of Minnesota,[1] and The Marine Biological Laboratory, Woods Hole, Massachusetts

The behavior of blue-green algae toward light has been examined by several investigators in the past, and it has been known for some time that these organisms carry out photosynthesis with the evolution of oxygen (Engelmann, 1883). The work of Emerson and Lewis on the participation of phycocyanin in the photosynthesis of *Chroococcus* has again increased the interest in the photosynthetic mechanism of blue-green algae. There are observations in the literature (Nakamura, 1938) which would indicate that certain algae, including several of the blue-greens, are able to oxidize sulfide to sulfur in the light. The interpretation of Nakamura's data is somewhat difficult, and we shall attempt to elucidate certain phases of this problem in a later paper.

Several years ago it was discovered that certain green algae, when incubated in molecular hydrogen, will undergo a change in their metabolic behavior (Gaffron, 1939). In the light these algae will now consume carbon dioxide with the simultaneous uptake of molecular hydrogen while the evolution of oxygen has ceased. To demonstrate this phenomenon, aerobically grown algae have to be incubated for several hours in darkness in an atmosphere of hydrogen before photoreduction of carbon dioxide by hydrogen can be observed in the light. This change of metabolism which involves the activation of a hydrogenase system has been designated as adaptation. It could also be shown that partial pressures of oxygen above 0.5 per cent or high light intensities can bring about de-adaptation, whereupon the organism will return to its normal aerobic metabolism. This work has been reviewed by Gaffron (1944) and by Rabinowitch (1945). Attempts have been made to adapt species of the blue-green alga *Oscillatoria* to such anaerobic conditions, but these experiments proved to be unsuccessful. (Gaffron, unpublished; summarized by Rabinowitch, 1945, p. 129.)

In this paper we shall report on the metabolism of a species of blue-green algae which can be adapted. In the dark and in the presence of molecular hydrogen a hydrogenase becomes active after several hours of incubation. On exposure to low light intensities the organism will absorb carbon dioxide and hydrogen (Table I), and thus behave like certain species of green algae which can be adapted anaerobically.

The first species in which we were able to demonstrate photoreduction was *Synechococcus elongatus* Näg. (Geitler, 1925), and we used a naturally occurring water bloom for our experiments. We have not been able to grow this organism

---

[1] This work was supported in part by funds from the Graduate School of the University of Minnesota.

and also were not able to find a comparable bloom the following year, and thus we had to discontinue our physiological studies of this species. We were fortunate to find a species of *Chroococcus* which exhibits the same physiological characteristics. This organism has been grown in pure culture and is available for further studies (Frenkel, 1949).

Two other blue-green algae were tested for their ability to carry out photoreduction. *Nostoc muscorum* was very kindly supplied to us by Dr. F. E. Allison, and a species of *Cylindrospermum* by Mr. R. Hecker. We were not able to demonstrate an active hydrogenase in either of these organisms; therefore, it appears that the existence of adaptive hydrogenase systems in algae is not restricted to several species or strains among the green algae, but seems to have a more widespread distribution. Until more is known about the distribution of this enzyme system in other groups of algae and in higher plants, it will be difficult to make any generalizations as to the physiological and evolutionary significance of this anaerobic type of metabolism.

TABLE I

Photoreduction by suspensions of Synechococcus elongatus, Näg.

Vessel (1). 4 ml. of 0.1 *M* Warburg bicarbonate-carbonate buffer No. 9 (Na:K = 2:1); gas atmosphere H_2; $k(H_2) = 1.34$

Vessel (2). 4 ml. of *M*/100 bicarbonate solution (Na:K = 2:1); gas atmosphere 4 per cent CO_2 in H_2; $k(CO_2) = 1.68$ (corrected for retention); $k(H_2) = 1.30$; temperature = 25° C.

Treatment		Duration in minutes	Pressure change in mm. per minute		$\frac{-\Delta H_2^*}{-\Delta CO_2}$	Remarks
	Light intensity in foot candles		Vessel (1)	Vessel (2)		
Dark	—	40	− .04	− .09		
Light	20	60	− .37	− .90	0.58	Incomplete adaptation of the hydrogenase system
Light	30	20	− .85	−1.25	1.97	
Light	30	40	−1.05	−1.50	1.90	
Light	30	50	−1.20	−1.53	3.0	
Light	62	10	−1.05	−1.45	3.1	
Light	100	10	− .35	−1.45	0.26	
Light	107	10	− .15	−1.35	0.1	Reversion to normal evolution of oxygen upon continued illumination at this light intensity

* Calculated from observed pressure changes in vessels (1) and (2).

MATERIALS AND METHODS

During the summer of 1947, one of the authors (E. B.) discovered a rather abundant occurrence of unicellular blue-green algae not far from Woods Hole, Massachusetts. These algae occur in small depressions filled with rain water on Angelica Rock, a small island in Buzzards Bay near New Bedford. Angelica Rock is frequented by a great number of gulls and terns, and thus parts of the island take on an almost white appearance from the droppings of the birds. The exposed rock consists mainly of granite which has weathered considerably and shows a series of small puddles which are about ½ to 3 feet wide, 1 to 15 feet long and ½ to 1½ feet deep. These water areas were almost completely exposed to the sun and the winds with very little protection from the walls of the rock. At the time we made our collection (August 28, 1948, 2:30 P.M.), the temperature of the water in the exposed depressions was 28° C. Some of the smaller depressions gave the appearance of having dried out, others were filled with a dense suspension of blue-green algae which gave a dark green appearance to the water. The green suspension which we collected proved to consist, on the basis of fresh weight, of 1 per cent of blue-green algae and was almost completely free of other organisms. The suspending medium at the time of collection had a pH of 8.65, but contained only traces of bicarbonate and carbonate, also only traces of sodium and chloride.

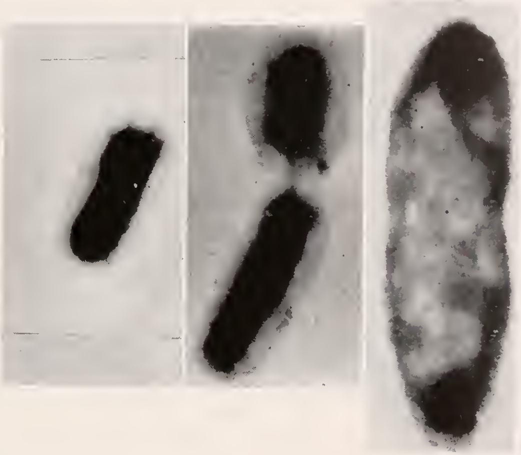


FIGURE 1. Electron micrographs of *Synechococcus elongatus* Näg. 1 a. Air dried cells. × 13,500; b. Cells treated for 24 hours with an aqueous solution of 0.2% osmium tetroxide. × 31,500 (electron microscope magnification × 15,000).

The bottom of the depressions was covered with a layer, several inches deep, of a very dark, loose mud which definitely smelled of hydrogen sulfide and gave a test for sulfide with lead acetate. This mud also contained unicellular blue-green algae as well as abundant protozoa, daphnias, and a variety of other organisms.

The species of *Chroococcus* mentioned previously can readily be grown on agar and in liquid media of similar composition as the mineral medium described by Emerson and Lewis (1942), (Frenkel, unpubl.).

Appearance of the blue-green algae (Fig. 1)

The algae which we collected appear to have no visible sheath as seen under oil immersion; under the electron microscope, however, a very delicate and narrow sheath is revealed. They occur either singly or in a linear row up to 4 cells. The single cells are 1 to 2 micra wide and from 1½ to 3 micra long. From descriptions given in the literature we are inclined to classify them under *Synechococcus* Nägeli 1849, and according to Geitler would classify them as *Synechococcus elongatus* Näg. These blue-green algae are gram negative and are associated with colorless bacteria which are mostly also gram negative.

Pigmentation

Dr. C. B. Van Niel, to whom we sent samples, pointed out to us the similarity of the visible absorption spectrum of our intact algae with that published for *Chroococcus* (Emerson and Lewis, 1942). On extraction with an 80 per cent acetone-water mixture followed by 100 per cent acetone, all the yellow pigments and the chlorophyll can be readily extracted. The chlorophyll fraction was purified chromatographically and its spectrum corresponded to that of chlorophyll *a*. The blue pigment is difficult to extract. The only way we have been able to obtain a small amount of soluble phycocyanin is by treatment of the algae with 0.1 *N* hydrochloric acid on a steam bath for 10 minutes. The blue pigment thus obtained gives an absorption spectrum with a protein band similar to that published for phycocyanin from blue-green algae by Svedberg and Katsurai (1929). We have found no evidence for the occurrence of Phycoerythrin in *Synechococcus elongatus.*

Measurement of photosynthesis and photoreduction

We have employed the conventional Warburg equipment for the measurement of gas exchange. As a light source we used a bank of 150 watt Mazda bulbs, whose intensity could be varied by means of a rheostat or by changing its distance from the experimental vessels. All measurements were made at 25° C.

To prepare the algae for measurements of gas exchange we first centrifuged the suspension at 100 times gravity for five minutes, which took down only a small fraction of the blue-green algae. The supernatant suspension was decanted and centrifuged at 2000 times gravity for ten minutes. The supernatant was discarded although it was still light green. The sediment was then resuspended in dilute bicarbonate solution or in a dilute bicarbonate-carbonate buffer (Warburg No. 9, Na : K = 2 : 1). The cells were washed once or twice more in these media and then made up to the proper volumes. We collected several gallons of suspension containing *Synechococcus* which were stored in a refrigerator at 3° to 5° C. Under these conditions of storage the algae remained healthy, and we did not observe any decline over a period of two weeks in their capacity to carry out photosynthesis or photoreduction when brought to room temperature.

RESULTS

A storage culture of these blue-green algae, when transferred to a dilute bicarbonate or a bicarbonate-carbonate buffer, will carry out photosynthesis at a constant rate for several days. In phosphate buffer, at pH 5.8 in equilibrium with 4 per cent CO_2 in air, the photosynthetic rate declines quite rapidly with time, and thus we have made all our measurements in bicarbonate solutions or bicarbonate-carbonate buffers. At light saturation (approximately 1000 foot candles) we obtained a quotient for $-\frac{\Delta O_2}{\Delta CO_2}$ of 1.08 ± .03 which agrees with most of the values for unicellular green algae (Rabinowitch, 1945, p. 33).

It was of interest to see if these blue-green algae could be adapted to utilize hydrogen for reduction of CO_2 in the light, as they had been found in a habitat favoring development of anaerobic organisms. All experiments were successful in which we attempted to adapt these organisms to such a metabolism. Comparable experiments with *Scenedesmus obliquus* (strain D–3) showed that it took longer for equally dense suspensions of the blue-green algae to become adapted than for the green alga *Scenedesmus*. However, once photoreduction had started it would continue for several days at low light intensities.

We have attempted to measure the $H_2 : CO_2$ quotient by a two vessel method using dilute bicarbonate and a carbonate-bicarbonate buffer. Later experiments, with the species of *Chroococcus* mentioned previously, have indicated that the rate of adaptation and of photoreduction is affected by the pH of the medium and thus we believe that we have obtained only an approximate measure of the $H_2 : CO_2$ quotient in this alga.

It is evident from Table I (carbonate-bicarbonate vessel) that the rate of hydrogen absorption increases with increasing light intensity until reversion to normal oxygen evolution occurs. The rate in the light is considerably higher than the rate of gas absorption in the dark, and thus demonstrates the existence of photoreduction in these algae. In these experiments we have no definite proof that CO_2 is reduced simultaneously with the absorption of H_2, in view of the pH sensitivity of the organisms. Gas analyses, however, in experiments with fully adapted *Chroococcus* suspensions have established a $H_2 : CO_2$ quotient in this alga which is close to 2. Only further studies will show if the observed changes in the quotient are significant and to which extent its actual value depends on the state of adaptation of the cells. It is logical that the quotient should change upon de-adaptation of the hydrogenase and should depend upon the state of reduction of the cells.

Adapted cell suspensions of *Synechococcus* and also of *Chroococcus* sp. differ in an interesting manner from those of *Scenedesmus* D–3. De-adaptation of the hydrogenase by increasing the light intensity is a gradual process in these blue-green algae (Table I), and re-adaptation can be brought about at low light intensities. In *Scenedesmus,* reversion to photosynthesis at higher light intensities is more sudden and re-adaptation requires incubation of the suspension in the dark.

SUMMARY

1. Enrichment cultures of *Synechoccus elongatus* Näg., a unicellular blue-green alga, carry out photosynthesis in a carbon-dioxide-bicarbonate buffer with a quotient of 1.08 ± .03.

2. This alga has a hydrogenase which can be activated anaerobically in the presence of molecular hydrogen. In the light, adapted algae can carry out photoreduction.

3. In contrast to *Scenedesmus*, activation of the hydrogenase can be carried out at low light intensities.

BIOLOGICAL

LITERATURE CITED

Emerson, R. and C. M. Lewis, 1942. The photosynthetic efficiency of phycocyanin in *Chroococcus,* and the problem of carotenoid participation in photosynthesis. *Jour. Gen. Physiol.,* **25** (4) : 579–595.

Engelmann, T. W., 1883. Farbe und Assimilation. *Botanische Zeitschrift,* **41**: 1–13, 17–29.

Frenkel, A., 1949. A study of the hydrogenase systems of green and blue-green algae. *Biol. Bull.,* **97** (2) : 261–262.

Frenkel, A. Photosynthesis and photoreduction by a species of *Chroococcus.* (In preparation for publication.)

Gaffron, H., 1939. Reduction of carbon dioxide with molecular hydrogen in green algae. *Nature,* **143**: 204–205.

Gaffron, H., 1944. Photosynthesis, photoreduction and dark reduction of carbon dioxide in certain algae. *Biol. Review,* **19**: 1–19.

Geitler, L. and A. Pascher, 1925. Die Süsswasserflora Deutschlands, Österreichs und der Schweiz. Heft 12. Cyanophyceae. Fischer, Jena.

Nakamura, H., 1938. Über die Kohlensäureassimilation bei niederen Algen in Anwesenheit des Schwefelwasserstoffs. *Acta Phytochimica,* **10** (2) : 271–281.

Rabinowitch, E., 1945. Photosynthesis. Chapter 6. The metabolism of anaerobically adapted algae. Interscience, N. Y.

Svedberg, T. and T. Katsurai, 1929. The molecular weights of phycocyanin and of phycoerythrin from *Porphyra Tenera* and of phycocyanin from *Aphanizomenon flos aquae. Jour. Am. Chem. Soc.,* **51**: 3573–3583.

PHOTOREACTIVATION OF ULTRAVIOLET LIGHT INJURY IN GAMETES OF THE SEA URCHIN STRONGYLOCENTROTUS PURPURATUS[1]

PATRICK H. WELLS AND ARTHUR C. GIESE

Department of Biological Sciences, Stanford University, California

The finding that visible light can reverse the deleterious effects of ultraviolet radiations (Kelner, 1949a; Prat, 1936; Whitaker, 1942) has resulted in numerous studies attempting to determine how widespread the phenomenon is and the possible mechanism of action of the ultraviolet and the visible light. The majority of these studies have been concerned with reactivation of ultraviolet killed organisms. Photoreactivation of the killing effect of ultraviolet light has been demonstrated on the bacterium, *Escherichia coli* (Kelner, 1949b; Novick and Szilard, 1949) and on the fungi, *Streptomyces griseus* (Kelner, 1949a), *Penicillium notatum, Saccharomyces cerevisiae* (Kelner, 1949b), *Ustilago maydis* (Snyder, unpublished), and others. The phenomenon appears to be basically similar in all these organisms. Exposure of ultraviolet inactivated cells to visible light results in a partial recovery of the lost viability.

There are also reports on the photoreactivation of injurious effects other than killing. Reactivation of ultraviolet induced delay in rhizoid formation in zygotes of the alga *Fucus furcatus* (Whitaker, 1942), photoreactivation of ultraviolet retarded adaptive galactozymase formation in *Saccharomyces cerevisiae* (Swenson and Giese, 1950), and photoreactivation of mutations in *E. coli* to bacteriophage resistance (Novick and Szilard, 1949; Kelner, 1949b) serve as examples. Here too, exposure to visible light after or during ultraviolet irradiation causes partial recovery from the ultraviolet injury.

The experimental conditions necessary to demonstrate photoreactivation in ultraviolet inactivated bacteriophage differ somewhat from the above. The inactivated phage particles alone can not be photoreactivated by visible light, but must be adsorbed onto bacteria before the phenomenon will occur (Dulbecco, 1950).

Blum and his co-workers reported photoreactivation in eggs of the sea urchin *Arbacia punctulata* (Blum, Robinson and Loos, 1949; Blum, Loos, Price and Robinson, 1949). Marshak studied photoreactivation in gametes and zygotes of this sea urchin (Marshak, 1949). He finds no photoreactivation of ultraviolet injury to either gamete alone, but that this does occur if the visible light follows fertilization. He analogizes this situation to that in bacteriophage.

The present study is concerned with photoreactivation in gametes and zygotes of the sea urchin, *Strongylocentrotus purpuratus*. In the literature on photoreactivation, work with monochromatic ultraviolet light is strikingly absent, yet the effects of various wavelengths may be quite different. Therefore, visible light reversal of injury caused to eggs by different monochromatic wavelengths of ultraviolet light

---

[1] Supported in part by grants from the Rockefeller Foundation.

has been attempted and the region of effective visible light defined. Effects of different dosages of ultraviolet radiations have also been studied. Photoreactivation has been attempted before and after fertilization of the injured gametes, and the effects of visible light on sperm investigated.

Materials and Methods

Sea urchins were collected at Moss Beach, California and later in the breeding season at the mouth of Malpaso Creek near Point Lobos, California, and brought to Stanford University where they were kept at 5° C. for two or three days during which time the gametes were used for experiments. The animals from the Malpaso Creek collecting station, which show a delayed breeding season, were often kept for several days in the aquaria of the Hopkins Marine Station at Pacific Grove, California before transport to Stanford University. There was little deterioration of gametes under these conditions of storage, and no detectable difference between material from the two collecting stations.

Eggs were obtained by cutting open a sea urchin to expose the gonads, rinsing with sea water, and taking some of the eggs which spew out when an ovary is slightly injured. Care was taken to avoid contact of the eggs with body fluids or any organs of the animal. The entire testes were cut out of the males and sperms were taken from these when required. Sperms were used in dilution of 1/400, or else insemination was accomplished by simply touching the tip of a dissecting needle to the testis, and then running this through the egg dish. Only gametes which showed almost 100 per cent fertilization and good early development were used for experiments. Sea water was obtained at the same places as were the urchins. It was filtered immediately and pH determinations showed that it did not change much on standing thereafter.

Dishes were made from the bottoms of new glass vials and were carefully tested to show that they supported normal development before being used in experiments. All operations except the irradiations with the monochromator were performed in a 13° C. constant temperature room. Monochromator irradiations were done at room temperature. Tests showed that such brief exposures to room temperature before fertilization had no detectable effect on the gametes.

The ultraviolet irradiation technique was the same as previously described (Giese, 1938a, b) and the eggs were handled in essentially the same manner. In essence, the source of radiations was a quartz mercury arc used with a natural quartz monochromator. The intensity of a desired wavelength of light was measured at the time of the experiment with a thermopile calibrated against a Bureau of Standards Standard Lamp.

After ultraviolet irradiation, the gametes were carefully screened from visible light, except for controlled exposure during photoreactivation. For this the brilliant light of a 100 Watt G. E. projection spotlamp was used. The lamp was 70 cm. from the eggs and the light passed through 20 cm. of water to remove heat rays and a Corning No. 3060 glass filter to remove short ultraviolet. This filter has a transmission from about λ3700Å through the visible (50 per cent transmission at λ4100Å), and allows good photoreactivation. Blue light from the monochromator (λ4350Å) was used in work with sperm.

Delay in cleavage of the zygotes (formed from union of ultraviolet irradiated gametes of one sex with normal gametes of the opposite sex) was taken as the criterion of injury, and reduction of this ultraviolet induced delay by visible light as photoreactivation. The time at which 50 per cent of an experimental sample of zygotes were divided was taken as the point of cleavage. The method routinely used for obtaining data on the per cent cleavage in a sample was to photograph it at intervals during the course of the experiment in essentially the same way as was done by Blum and Price (1950a, b). This was done with a Leica camera through a Microibso attachment, using an ordinary Leitz monocular microscope with a Leitz No. 2 objective. This gives a field large enough to include one or two hundred eggs in each photograph. The microscope light was filtered through a Corning No. 3480 Glass filter which transmits only to λ5500Å in the visible (50 per cent transmission at λ5700Å). The filter effectively eliminated photoreactivation from this source and a 5 cm. water cell removed any heat. Exposures of two and one-half seconds on Kodak Microfile film were adequate under the lighting conditions used. The negatives obtained in this way were projected with an enlarger and counts made from them. This method gave a permanent record which could be read at leisure. For critical experiments, the counts were made a number of times and their results were found to be quite repeatable. Experiments were usually repeated three times, and dark and light controls were run on each experiment.

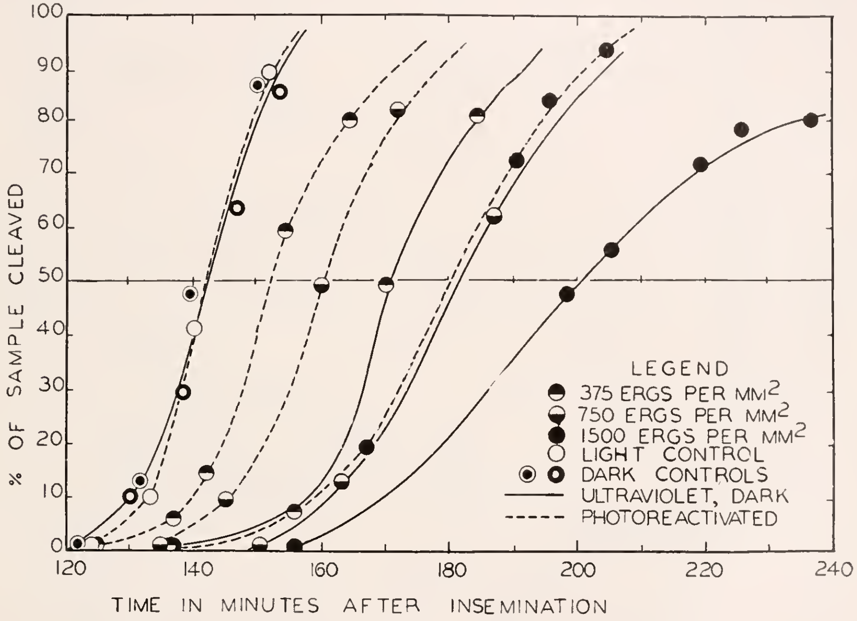


FIGURE 1. Delay in cleavage of sea urchin eggs injured with various dosages of ultraviolet wavelength 2450Å, with and without photoreactivation. All irradiations completed before fertilization. Visible light exposures are the same in each case. Dosage series at λ2537Å and λ2654Å show similar cleavage characteristics.

EXPERIMENTAL

In the first group of experiments, the effects of series of dosages of ultraviolet light at λ2450Å, λ2537Å, and λ2654Å were determined. Eggs were irradiated with the indicated dosages of ultraviolet, then exposed to white light from the G. E. spotlamp for one-half hour, and fertilized with normal sperm. Each series was done with eggs from a single female and sperm from a single male. Data on cleavage were determined as described above, plotted, and the points of 50 per cent cleavage determined. Figure 1 shows the cleavage characteristics of such a series at λ2450Å. The points of 50 per cent cleavage from Figure 1 and from similar series of dosages at λ2537Å and λ2654Å are plotted in Figure 2. In each of these figures, solid lines represent dark controls and ultraviolet injured eggs. The dotted lines represent visible light controls and photoreactivated samples.

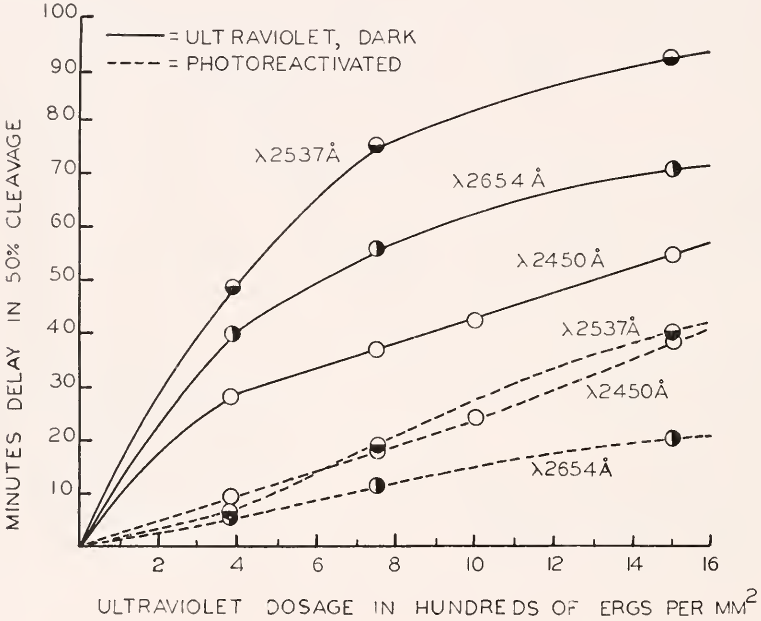


FIGURE 2. Photoreactivation of injury to eggs caused by wavelengths 2450Å, 2537Å, and 2654Å. Times of 50 per cent cleavage of the samples of eggs are compared here. Visible light dosages were the same in each experiment. All irradiations were completed before insemination.

Complete dosage series were not run on other wavelengths, but representative dosages were tested. In Table I some of these data are compared, data from Figure 2 being included. Visible light exposure was held constant for all of these experiments.

Photoreactivation occurred for all wavelengths tested and for each trial at any wavelength. Comparison of efficiencies of the process at different wavelengths is difficult because the wavelengths of ultraviolet light vary in the amount of injury caused by a given exposure. The action spectra for ultraviolet light injury to sea urchin gametes have been previously described (Giese, 1946). There is also varia-

TABLE I

Representative photoreactivation data from various wavelengths of ultraviolet light, keeping the visible light dosage constant. Controls are taken as zero delay.

Wavelength of ultraviolet, in Angstrom units	Dosage of ultraviolet, in ergs/mm.^{2}	Cleavage delay, in minutes; ultraviolet	Cleavage delay, in minutes; photoreactivated	Per cent photoreactivation
Control	0	0	0	0
2450	750	37	18	51
2450	1500	55	18	31
2537	750	76	19	75
2537	750	36	6	83
2537	1500	93	40	57
2654	750	56	11	80.5
2654	750	51	14	72.5
2654	1500	71	20	71.7
2804	750	90	26	71
3025	750	37	11	70
3130	6000	14	2	86

tion in the sensitivity to ultraviolet injury in eggs from different females. It seems, however, from the data available (Figure 2 and Table I), that there is strikingly less reactivation from λ2450Å injury than from injury by other wavelengths. Furthermore, there is a marked unilateral effect in raising of the membrane at

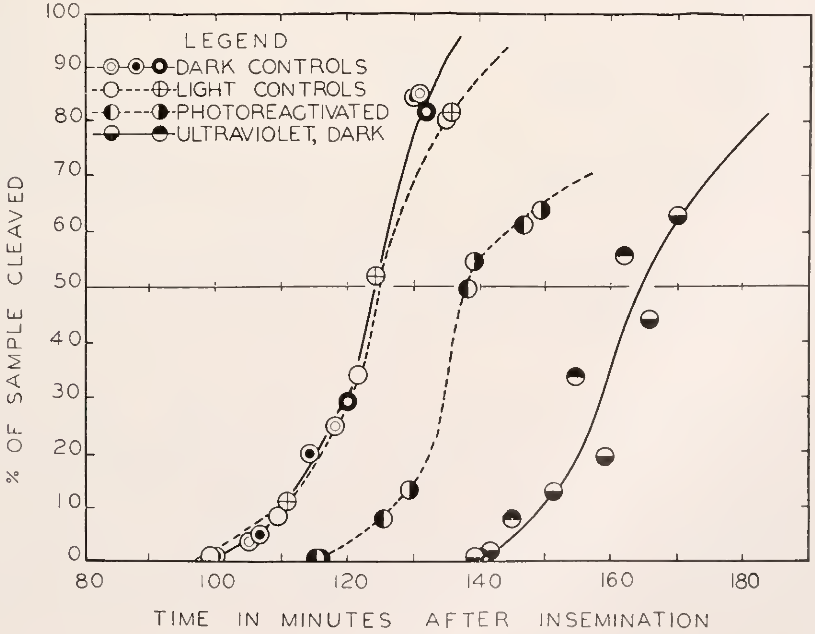


FIGURE 3. Photoreactivation of zygotes formed from eggs injured by 750 ergs/mm.^{2} of λ2654Å ultraviolet light. Visible light exposure of ⅓, used in the experiments of Figure 1 and Figure 2, was immediately after fertilization. Photoreactivation of 65.5 per cent was obtained.

λ2450Å which is not usually apparent at other wavelengths. This suggests that injury from superficially absorbed ultraviolet light is not so readily photoreactivated as injury caused by wavelengths which are more strongly absorbed by nucleoproteins. More data are necessary for a complete analysis of this situation.

In all of the experiments discussed above, eggs were photoreactivated with one-half hour exposures under the G. E. spotlamp, then fertilized with normal sperm. This was found by experimentation to be the minimal dosage which would give an effect reasonably near maximal. If a Corning No. 3389 glass filter was substituted for the Corning No. 3060 filter routinely used on the reactivating lamp, photoreactivation was greatly reduced. The Corning No. 3389 filter transmits only wavelengths longer than λ4100Å (50 per cent transmission at λ4300Å). It has been shown on other organisms that the spectral region λ3660Å to λ4300Å is usually most effective in photoreactivation (Dulbecco, 1950; Kelner, 1950). Our experiments show that in Strongylocentrotus, wavelengths shorter than λ4300Å are most effective.

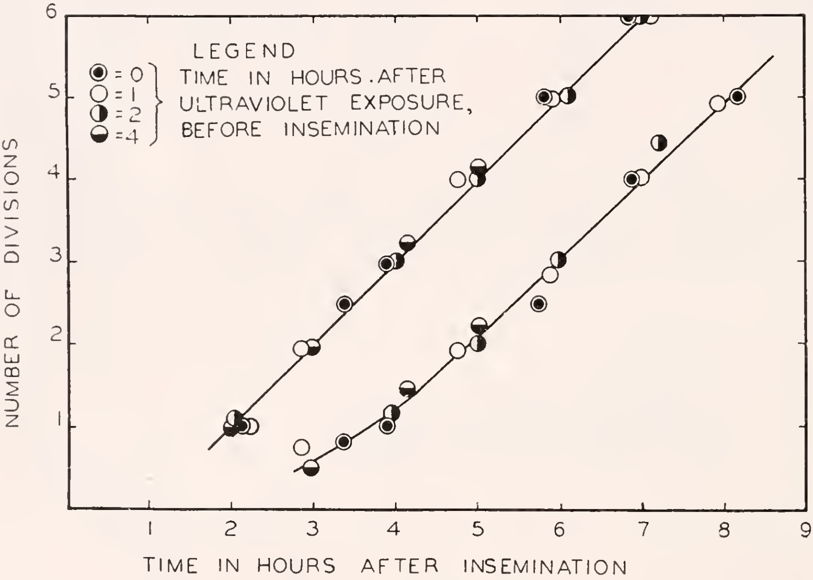


FIGURE 4. Comparison of cleavage rates of sea urchin eggs injured by ultraviolet light, then kept in the dark for various periods of time before insemination. Two experiments are shown.

That fertilization prior to photoreactivation is not required is indicated by all of the experiments thus far described. It was found, however, that after fertilization of injured gametes, photoreactivation of the resulting zygotes was somewhat more easily accomplished. Visible light dosages of ten minutes sufficed to cause reactivation of the same magnitude as was caused by thirty minutes exposure on the unfertilized eggs. Figure 3 shows the results of one experiment on reactivation of zygotes.

Eggs of Strongylocentrotus do not show any measurable increase or decrease in amount of delay caused by an ultraviolet exposure, when they are kept in the dark in sea water. This was experimentally shown by irradiating samples of eggs with ultraviolet light from a sterilamp (arbitrary exposure of sixteen seconds), and fertilizing portions of the samples at intervals after the irradiation. Samples were followed through the sixth division in order to detect any change in rate of cleavage caused by the delay in fertilization. Portions of each sample were inseminated immediately after irradiation, and at one, two, and four hours thereafter. Figure 4 shows the results of two such experiments. Note that the delay of cleavage for all sample portions of irradiated eggs is practically identical.

Sea urchin sperm are much more sensitive to ultraviolet light injury than the eggs. Fifty ergs of λ2654Å suffices to cause a delay in cleavage of 80 to 100 minutes in zygotes from such injured sperm. An action spectrum has been worked out which shows that this wavelength is near the peak of efficiency for ultraviolet injury to sperm, and a detailed comparison of ultraviolet effects on eggs and sperm has previously been made (Giese, 1946, 1949). The most obvious effect of ultraviolet light injury to sperm is retardation in the cleavage of zygotes resulting when injured sperm are used to fertilize eggs. Even after quite large dosages of ultraviolet, the sperm remain motile and activate the eggs.

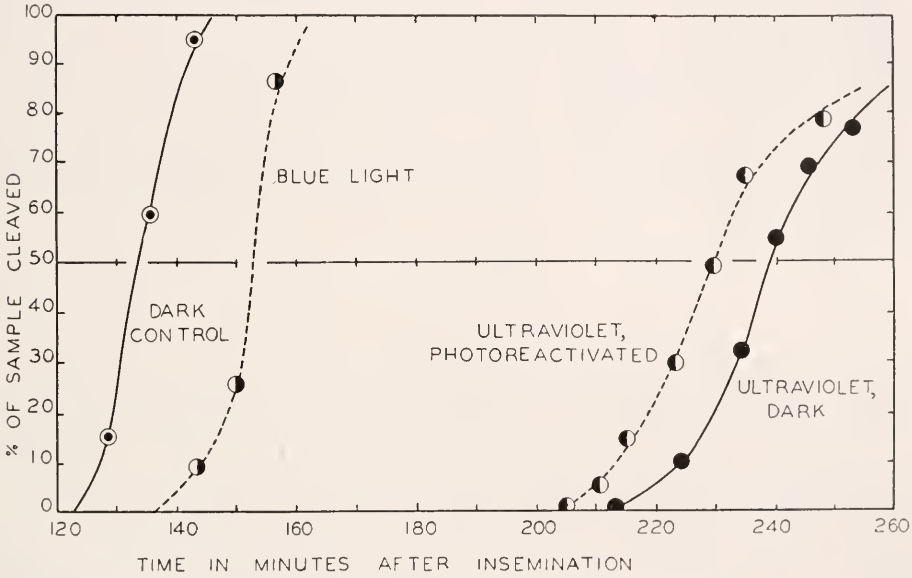


FIGURE 5. Photoreactivation of ultraviolet injured sperm. Sperm were injured with 50 ergs/mm.^{2} of λ2654Å ultraviolet, photoreactivated with 4086 ergs/mm.^{2} of λ4350Å blue light, then used to fertilize normal eggs. Cleavage delay in the resulting zygotes is shown. Note the retardation of cleavage in the blue light control.

Visible light is also very harmful to sperm. Its effects will be reported in detail elsewhere, but it is necessary to present some of the data here, in connection with photoreactivation in sperm. The visible light injury is qualitatively different from ultraviolet light injury. Sperm exposed to visible light become immotile and fail to activate the eggs. Sensitivity varies with gametes from different individuals. Usually the dosage which will kill nearly 100 per cent of the sperm is between 20,000 ergs/mm.^{2} and 40,000 ergs/mm.^{2} for monochromatic blue light of λ4350Å (the wavelength used in photoreactivation experiments with sperm). Sublethal dosages cause a delay in cleavage of the zygotes formed from sperm so injured. This delay is of a lower order of magnitude, however, than the ultraviolet induced delay.

The injury caused by ultraviolet light on sperm appears to be different from the injury caused by visible light. Visible light of low intensity might, therefore, cause photoreactivation of ultraviolet induced delay in cleavage, even though the visible light is itself injurious.

In order to test this, sperm suspensions of 1/400 were irradiated in a quartz vessel in the monochromator with 50 ergs/mm.^{2} of λ2654Å ultraviolet, followed by sublethal dosages of blue λ4350Å (4000 to 5000 ergs/mm.^{2}), appropriate controls being run. Normal eggs were then fertilized with these sperm, and the time of cleavage determined. Photoreactivation was in fact demonstrated. Figure 5 shows one such experiment. This was repeated three times with similar results. Note the retardation of cleavage in the blue light control. In spite of this injury, and in spite of the relatively low dosage of visible light used, a photoreactivation of ten minutes is observed.

Discussion

Most previous studies on photoreactivation have been with a sterilamp as the source of ultraviolet radiations. Such sterilamps give off mixed wavelengths of ultraviolet with maximum intensity at λ2537Å. The data presented here indicate that cleavage delay caused by any of the wavelengths of ultraviolet tried is photoreversable, but that λ2450Å injury is less so than injury by wavelengths longer than this. λ2450Å is strongly absorbed by surface proteins, as indicated by the unilateral effect obtained in raising of the membranes when eggs are injured by this wavelength. Several wavelengths tested are much more selectively absorbed by nucleoproteins. It is suggested, then, that injury to nucleoproteins is most strongly photoreactivated. This should be more adequately investigated. It is of interest to note that wavelengths λ2654Å and longer do not produce appreciable amounts of ozone. Therefore, the reversable injury is not due to ozone formed by the ultraviolet light.

Marshak (1949) has found that in Arbacia the injured gametes cannot be photoreactivated, but that zygotes formed from injured gametes are quite sensitive. The data on Strongylocentrotus do not agree with these findings. Either there is a great difference in these two sea urchins with respect to photoreactivation, or the dissimilarities must be accounted for by differences in technique. Since the injured eggs in Strongylocentrotus are less sensitive than zygotes formed from them, perhaps the visible light illumination periods used on Arbacia eggs were too short to cause measurable photoreactivation. Failure of ultraviolet injured sperm to be photoreactivated may have been due to visible light injury to them.

It has been suggested (Novick and Szilard, 1949) that the injurious effect of ultraviolet light may be due to the formation of some sort of poison. Since photoreactivation is never complete and is possible only for a time after ultraviolet irradiation, they suggest that the poison has two forms, one stable to visible light and one photolabile. As time passes after injury, the second is largely transformed to the first. Hertel (1905) discussed the possibility of the ultraviolet effect being due to the formation of a poison. He found that if one of a pair of blastomeres was irradiated, the second would be effected, provided the cleavage plane had not yet entirely separated the two. The second blastomere, he argued, was affected by some toxic substance (poison) produced in the first and diffusing into the second.

If poison production is, in fact, the mechanism by which cells are injured by ultraviolet light, this poison must be of a size or form which prevents diffusion through the cell membrane. Hertel's experiment works only if the cleavage plane does not completely separate the blastomeres. Photolyzed eggs added to normal ones do not in any measurable way affect their cleavage. Ultraviolet light injured eggs of Strongylocentrotus do not show recovery, even after standing for hours in sea water. If the injurious substance were able to diffuse into or out of the cell through the membrane, it should certainly be demonstrable by these experiments.

Synthetic processes of the cell seem to be quite sensitive to ultraviolet radiation injury (Giese, 1950; Swenson and Giese, 1950). It can be hypothesized that the photoreversable effects of these radiations, such as killing, inhibition of adaptive enzyme formation, and cleavage delay, are all manifestations of a basic disruption of the synthetic processes of the cell. Failure to obtain complete photoreactivation suggests a multiple effect of ultraviolet light.

SUMMARY

1. Ultraviolet radiation induced cleavage delay in eggs of the sea urchin *Strongylocentrotus purpuratus* can be reduced by exposure to visible light before fertilization.

2. Photoreactivation by visible light occurs in injury by any of the wavelengths of ultraviolet light which we tested: λ2450Å, λ2537Å, λ2654Å, λ2804Å, λ3025Å, λ3130Å. The phenomenon is less pronounced at λ2450Å than at the other wavelenths tried.

3. Wavelengths shorter than λ4300Å are most effective in photoreactivation. The minimum visible light exposure giving an effect near maximal is determined.

4. Zygotes formed from ultraviolet injured eggs are more readily photoreactivated than the unfertilized eggs.

5. Ultraviolet irradiated eggs show no increase or decrease in injury when kept in the dark for several hours before fertilization.

6. Visible light is injurious to sperm.

7. Sperm injured by ultraviolet radiations can be photoreactivated, even though the visible light is itself harmful.

8. The results are compared with photoreactivation data in the literature and discussed with reference to possible mechanisms of action for the phenomena observed.

We are indebted to Dr. L. R. Blinks and Dr. R. L. Bolin for many courtesies extended to us at the Hopkins Marine Station, and especially to Dr. C. S. Yocum for help in obtaining suitable biological material.

LITERATURE CITED

Blum, H. F., J. C. Robinson and G. M. Loos, 1949. Effects of ultraviolet radiation on the rate of cell division in Arbacia eggs, and the enhancement of recovery by visible light. *Anat. Rec.*, **105**: 524.

Blum, H. F., G. M. Loos, J. P. Price and J. C. Robinson, 1949. Enhancement by "visible" light of recovery from ultraviolet irradiation in animal cells. *Nature*, **164**: 1011.

Blum, H. F. and J. P. Price, 1950a. Delay of cleavage of the Arbacia egg by ultraviolet radiation. *J. Gen. Physiol.*, **33**: 285–304.

Blum, H. F. and J. P. Price, 1950b. Time relationships in the cleavage of the normal fertilized egg of *Arbacia punctulata*. *J. Gen. Physiol.*, **33**: 305–310.

Dulbecco, R., 1950. Experiments on photoreactivation of bacteriophages inactivated with ultraviolet radiation. *J. Bact.*, **59**: 329–347.

Giese, A. C., 1938a. The effects of ultraviolet radiation of λ2537Å upon cleavage of sea urchin eggs. *Biol. Bull.*, **74**: 330–341.

Giese, A. C., 1938b. The effects of ultraviolet radiations of various wavelengths upon cleavage of sea urchin eggs. *Biol. Bull.*, **75**:238–247.

Giese, A. C., 1946. Comparative sensitivity of sperm and eggs to ultraviolet radiations. *Biol. Bull.*, **91**: 81–87.

Giese, A. C., 1949. Conference on problems of general and cellular physiology relating to fertilization. I. Activation of eggs, fertilization and early development as affected by ultraviolet rays. *Amer. Nat.*, **83**: 165–183.

Giese, A. C., 1950. The action of ultraviolet radiation on protoplasm. *Physiol. Reviews* (in press).

Hertel, E., 1905. Über die Einwirkung von Lichtstrahlen auf den Zellteilungsprozess. *Zeitschr. fallg. Physiol.*, **5**: 535–565.

Kelner, A., 1949a. Effect of visible light on the recovery of *Streptomyces griseus* conidia from ultraviolet irradiation injury. *Proc. Nat. Acad. Sci.*, **35**: 73–79.

Kelner, A., 1949b. Photoreactivation of ultraviolet-irradiated *Escherichia coli*, with special reference to the dose-reduction principle and to ultraviolet-induced mutation. *J. Bact.*, **58**: 511–522.

Kelner, A., 1950. Action spectra for photoreactivation. *Bact. Proceedings*, 1950: **53**.

Marshak, A., 1949. Recovery from ultra-violet light-induced delay in cleavage of Arbacia eggs by irradiation with visible light. *Biol. Bull.*, **97**: 315–322.

Novick, A. and L. Szilard, 1949. Experiments on light-reactivation of ultra-violet inactivated bacteria. *Proc. Nat. Acad. Sci.*, **35**: 591–600.

Prat, S., 1936. Strahlung unt antagonistische Wirkungen. *Protoplasma*, **26**: 113–149.

Snyder, J. B., personal communication.

Swenson, P. A. and A. C. Giese, 1950. Photoreactivation of galactozymase formation in yeast. *J. Cell. Comp. Physiol.* (in press).

Whitaker, D. M., 1942. Counteracting the retarding and inhibitory effects of strong ultraviolet on Fucus eggs by white light. *J. Gen. Physiol.*, **25**: 391–397.

CYST-FORMATION IN AEOLOSOMA HEMPRICHI (EHR)

HENRIETTE HERLANT-MEEWIS

Institute of Biologie of Brussells and University of Montreal

Cyst-formation has not often been observed in the Oligochaeta. It was observed for the first time by Beddard (1905a, 1905b) for the species *Aeolosoma hemprichi* Ehr. It was his opinion that cyst-formation appearing at the beginning of winter is due to the decrease of the temperature. According to Vejdowsky (1884), this phenomenon is a resting stage following a long period of asexual reproduction: while Dehorne (1916) considers it to be consecutive to sexual reproduction, which effectively occurs in the months of November and December, Beddard (1889) and Stolc (1889).

The opportunity to study this phenomenon occurred during my sojourn in Canada, at the University of Montreal. The author wishes to express her indebtedness to the Board of Trustees of this Institution: to Dr. Henri Prat, Director of the Institute of Biology and to Father O. Fournier, Assistant-Director, who helped us greatly in the accomplishment of this work. I wish to acknowledge the active collaboration of Miss Marthe Demers.

Scope of the Work

Our concern is cyst-formation in *Aelosoma hemprichi* Ehr, induced experimentally in order to follow the successive stages from the beginning of the phenomenon until the emergence of the encysted worm. These observations also permit a study of the causes that determine cyst-formation in natural surroundings, and the related factors and the importance of the phenomenon in the biological cycle of the worm.

Materials and Technique

The genus Aeolosoma

While studying the fresh-water fauna of some brooks in the region of Montreal, the three following species of Aeolosomatidae were encountered: *A. hemprichi, A. haedleyi* and *A. tenebrarum*. Their systematic position will be studied in another report. This paper will be restricted to the species *A. hemprichi*. The genus Aeolosoma is easily recognized by the ciliated prostomium and the lipoidic inclusions in the skin. The color of these inclusions varies with the species: in *Aeolosoma hemprichi* they are red. The presence of setae and of nephridia is the only means of recognizing metamerisation, since Aeolosomatidae possess neither intersegmentary septa, nor nerve cord with segmentary ganglia.

The transparency of the worm facilitates the study of the internal organs, especially the components of the digestive tract: the ciliated prostomium, the muscular pharynx, the narrow esophagus and the large stomach which gradually merges with the intestine.

This worm reproduces asexually very rapidly. The laws governing this scissiparity have been studied previously (Meewis, 1933). They may be summarized as follows:

(1). Every individual is composed of six seta-bearing segments, the pharyngeal, the esophageal and the four stomacho-intestinal segments. As soon as these have appeared, the pygidium becomes longer and divides into two parts: a small anterior one which will regenerate the pygidium and a longer posterior one which becomes a new zoid. This mode of scissiparity was previously termed "pygidial budding."

(2). When the pygidial bud is formed, it has no seta. However, the first groups of setae soon appear, and progressively, as the bud grows longer, the following groups are formed. When the bud reaches the five seta-bearing segment, all the setae are practically equidistant. Simultaneously, the cephalic zone develops and the pharyngeal setae appear at the anterior part of the bud. These are at first close to the esophageal setae, but as the esophagus grows longer they separate from each other. The zooid is then complete, and possesses a head, six seta-bearing segments and a pygidium capable of budding.

(3). The initial bud composed of six segments will now present a secondary bud.

(4). When cephalization is complete, the initial bud separates from the chain bearing the buds to which it gave rise. Thus, true scissiparity which divides an individual into two parts, more or less equal, and which characterizes fresh-water Oligochaeta, does not exist in this species. By means of an accelerated process, blastogenesis takes place exclusively by pygidial budding.

Aeolosoma hemprichi, represented in Plate I, Figure 1, is composed of an initial individual followed by the buds formed by its pygidium. It corresponds to the formula 6/0/3/6/0, which represents the number of seta-bearing segments of each successive zooid.

This pygidial budding forms chains comprising from two to six individuals or zooids, and of a mean length of 2 mm.

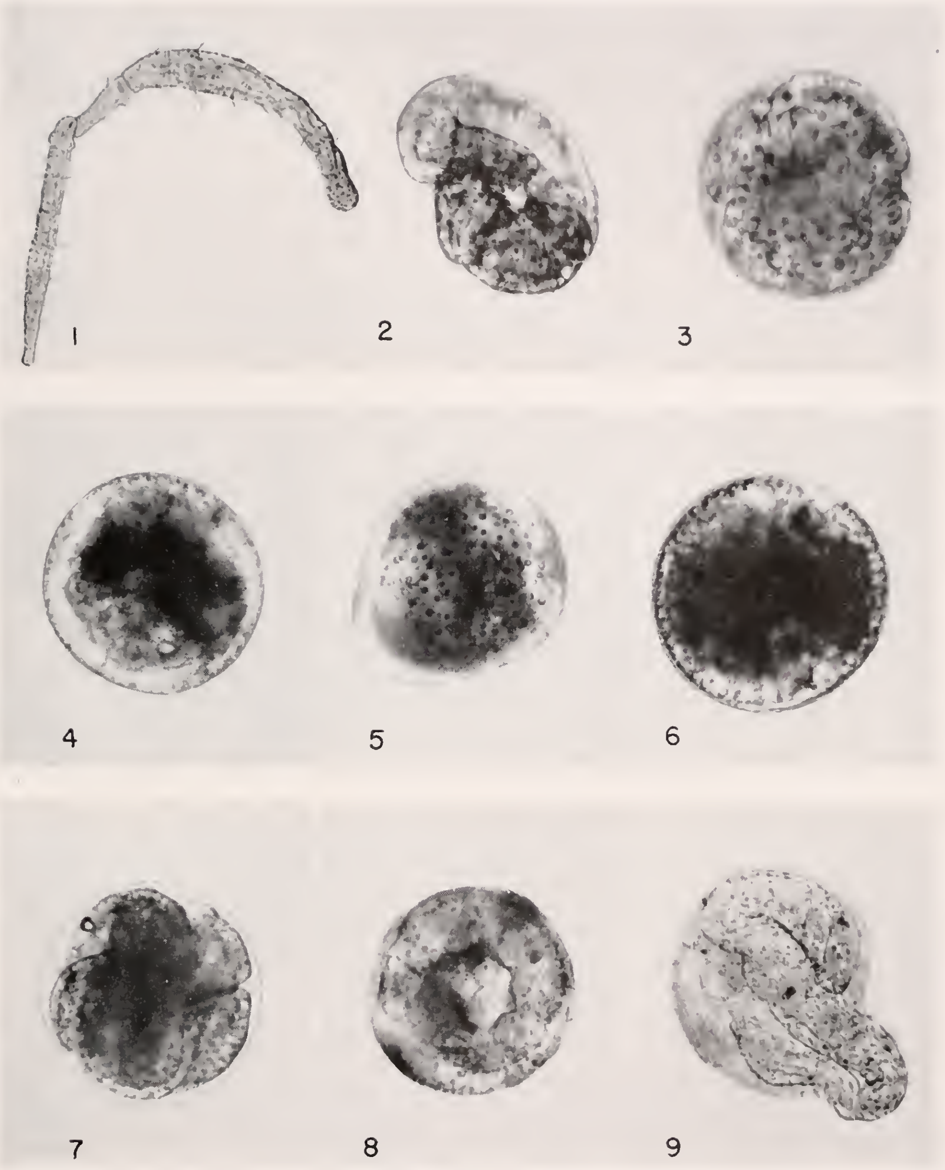
When environmental conditions are stable, pygidial budding occurs regularly and rapidly. The rate of growth varies with the amount of food available and the temperature.

In order to study the influence of environmental conditions on the laws of scissiparity, some cultures were kept at different temperatures ranging from 11° C. to 29° C., whereas the stock culture was kept in front of the laboratory window. Because of a sudden cold spell in the middle of the winter this stock culture was submitted to a temperature of 6° C.–7° C. for about 10 days and it was at this time that the first cyst-formation was noticed.

Description of the cyst

The cysts are generally spherical and of variable size, measuring from 175 to 250 microns in diameter; some may have irregular forms. The worm is easily visible through the thin and completely transparent membrane. The skin of the worm is in close contact with the cyst-membrane, the latter appearing as though spotted with red. The digestive tract, which is very opaque, is joined to the skin by means of mesenteric filaments.

Within the cyst, in cold surroundings, the worm is motionless and its contours difficult to see, though the peristaltic movements of the intestine make orientation



Explanation of Plate I

1. *Aeolosoma hemprichi:* Photograph of a live normal worm; 2. Pre-cystic stage; 3. Beginning of cyst-formation; 4. Quiescent cyst: equatorial view; 5. Quiescent cyst: tangential view; 6. Beginning of activation; 7. Activating cyst; 8. Pre-emergence stage; and 9. Emergence stage.

possible. When seen through the microscope, because of the heat emitted by the lamp, the worm turns around within the cyst. When this occurs its skin slides against the cyst membrane making these two tissues distinctly visible.

Plate I, Figures 4 and 5 show a cyst at 5° C.; the first one being a medium (equatorial); the other a tangential view (polar), shows the red pigments of the skin, the cyst membrane being quite transparent. Plate I, Figure 6 shows that the space between the cyst membrane and the worm is increased by its movements, as a result of the heat emitted by the lamp.

Cyst formation

When the first cysts were observed at the beginning of December, 1949, the culture still contained free living worms on which we were able to study cyst formation. These free worms present the following aspect: the chains are short and composed of two zooids corresponding at the most to the formula 6/1, *i.e.*, a complete anterior zooid of six seta-bearing segments, followed by a small non-segmented pygidial bud. According to our previous observations, this formula is the simplest one applying to this species. These individuals become bulky and opaque because of the accumulation of refractive granules around the digestive tract. Simultaneously, their movements slow down; whereas normal worms, because of their ciliated prostomium, move about rapidly in a way resembling that of rhabdocoela. Worms submitted to low temperatures crawl, change form, and evidently prefer regions rich in organic debris. While crawling and turning about they secrete a heavy mucus which hardens to form the cyst membrane.

In Plate I, Figures 2 and 3 show two stages of this phenomenon. In the first one, secretion of the mucus has started, although the worm can still stretch itself and move around. In the second case, the worm is imprisoned in its mucus which hardens and forms a shell-like covering.

Activation of the cyst

When a cyst is submitted to a temperature of 18° C.–20° C., its appearance changes rapidly. The worm turns around continuously while its head and pygidium, which were indistinct, become visible again. At the same time, the worm recovers its initial transparency; the refractive granules which surrounded the digestive tract disappear progressively.

Nevertheless, during the first few days, the worm (as shown on Plate I, Figure 6) is still as bulky as at the time of the formation of the cyst. After 8 to 10 days it reaches another stage in its transformation. It grows and bears new segments, following the laws of normal scissiparity. It becomes totally transparent and in every way resembles a free-living worm. Simultaneously its movements become very rapid, permitting it to turn over very quickly within its cyst. The growth can be followed by the study of the seta and this is rendered possible by the transparency of the cyst membrane. After twelve days, generally, at this temperature (18° C.–20° C.) the worm endeavors to free itself. In certain cases, emergence from the cyst seems easy enough: the membrane is first slightly ruptured, the worm then pushes its pygidium out through this opening and the remainder of its body follows. This phenomenon can take place within half an hour or sometimes necessitates several hours. It may happen that the cyst is covered with foreign matter or with a heavy coating of bacteria. When this occurs the worm cannot easily break through its cyst membrane and it may remain imprisoned for several days. In this case, its growth progresses as if it were free, producing new zooids, and thus there may be several chains within the cyst. This would explain the exceptional finding of three worms within a single cyst. But when the worm emerges under normal conditions it is generally composed of two zooids answering to the formula 6 3, *i.e.*, the chain is composed of a complete anterior individual with six seta-bearing segments and a three-segment pygidial bud.

It does not seem that emergence is caused by the action of a corrosive substance secreted by the worm, but is purely mechanical.

As soon as the worm is free it grows normally, its growth being regulated by the environmental temperature and the quality of the food present. It may live normally without eating for several days, but if the fasting persists, once the reserve substances have been used up, the worm grows thin and its coloring disappears. The effect of fasting on these animals has been previously described.

Plate I, Figures 7, 8, and 9 show three successive stages under heat activation. The worm appearing in Figure 5 is still bulky but its outline is quite distinct. In Figure 8 the worm has reached the pre-emergence stage and in Figure 9 it is emerging. We can observe, in the emerged part, the limit existing between the two zooids which make up the chain.

We can summarize the successive stages of cyst formation and emergence in *Aeolosoma hemprichi* as follows:

(1). Normal worm: (N.W.) the chains are composed of 2 to 6 individuals, the animal is transparent and moves rapidly.

(2). Depressed worm: (D.W.) the chains are of normal length but the movements are slower.

(3). Pre-cystic stage: (P.C.) the chains are made up of 2 individuals, at the most. The worm is bulky and opaque, its movements are very slow.

(4). Cyst-formation: (C.F.) the worm turns around in the secreting cyst.

(5). Quiescent cyst: (C.) the worm is motionless and its contours are indistinct.

(6). Activating cyst: (A.C.) the worm moves around and becomes transparent.

(7). Pre-emergence stage: (P.E.) the very active worm lengthens and tries to rupture the cyst.

(8). Emergence: (E.) the worm ruptures the cyst.

EXPERIMENTAL STUDY OF THE FACTORS DETERMINING CYST-FORMATION

Effect of low temperatures

No cyst is formed in cultures which are not subjected to low temperature from June to December: the worms undergo asexual reproduction.

Three groups of two hundred and fifty worms each were taken from a culture maintained at 18° C. and placed in an infusion rich in microorganisms. These cultures were then kept at three temperatures: 3° C., 6° C. and 11° C. The results are summarized in Table I.

These results show that:

1. At 3° C., the movements of normal worms are retarded and only a certain number of them attain the cystic stage. But these individuals seem less bulky and

less opaque than individuals at normal pre-cystic stages. The majority of them resemble fasting worms, as described earlier.

2. At 6° C., slowing of movements is followed by the formation of a normal pre-cystic stage. The first cysts appear after the worms have been kept about 15 days at this temperature. About 50 per cent of them, however, do not go through cyst-formation and remain in a pre-cystic stage for two months.

TABLE I

Duration: days	0	3	7	15	30	60
Exp. at 3° C.	N.W.	D.W.	A few P.C.	A few P.C.	A few P.C.	A few P.C.
Exp. at 6° C.	N.W.	D.W.	P.C.	First C.	Many C.; remainder P.C.	Many C.; remainder P.C.
Exp. at 11° C.	N.W.	D.W.	D.W.	D.W.	D.W.	D.W.

N.W.—normal worm; D.W.—depressed worm; P.C.—pre-cystic stage; C.—cyst.

3. At 11° C., activity is reduced and asexual reproduction is slower; the worms do not reach the pre-cystic stage, the long chains persist and the growth is similar to the one observed during summer months at the same temperature.

We can conclude as follows: (1) The optimum temperature for cyst-formation appears to be about 6° C. (2) The cysts are formed after about 15 days at a low temperature, but this value differs with each individual of the culture. (3) A low temperature is not alone responsible for cyst-formation, since this phenomenon takes place in part of the population at 6° C., and does not affect worms kept at 3° C.

Effect of food

To study those conditions besides temperature which must operate in order to produce cyst-formation, some worms were taken from a culture kept at 18° C. and rich in nutritive substances, and were placed in pure water at 3° C. and 6° C. At both temperatures the movements of the animals were slowed down but pre-cystic stages did not appear; after fifteen days the worms were either dying or dead.

Effect of the combined factors of temperature and food

Different combinations of these two factors were studied so as to determine precisely the role of the nutritive medium during the different stages of cyst-formation.

Two hundred and fifty normal worms were placed at 6° C. in a rich nutritive medium; when they reached the pre-cystic stage, they were divided into two groups: one was kept in the same conditions of temperature and food, while the other was placed in pure water at the same temperature. After seven days, the worms from the first group were encysting while those from the second group were nearly all dead, none having reached the cyst stage. So that besides being necessary for cyst-formation, food is also necessary after the pre-cystic stage.

The final experiments help to explain the results first obtained.

1. At 3° C., the microorganisms on which the worms feed no longer multiply actively and the medium becomes poorer in nutritive substances up to a stage where no cyst-formation can take place. Therefore, if this medium is renewed periodically, to supply sufficient food, normal pre-cystic stages will appear after 15 days and the first cysts within a month. Cyst-formation can take place at 3° C. provided the culture medium is constantly replenished; however, this phenomenon proceeds more slowly than at 6° C.

2. We have seen previously that if cultures which are kept at 6° C. (where cyst-formation is possible) are not maintained at a high nutritive level, a certain number of the worms will remain in a pre-cystic stage; these individuals thrive because enough food is present, but it is impossible for them to transform into cysts. Such pre-cystic worms, taken from a culture where no cyst-formation had resulted after one month at a low temperature, were placed in a rich nutritive medium: three days later, cysts appeared.

The influence of food is therefore obvious; the worm must be able to accumulate reserves during the stages preceding cyst-formation. The elaboration and the nature of these reserves will be studied in a future paper. The worm is especially in need of these reserves during its encysted and emergence stages.

Discussion and Conclusions

The phenomenon of cyst-formation cannot be considered solely as a resting stage occurring after a long period of asexual reproduction as proposed by Vejdowsky, for no cysts were formed in cultures kept in winter at temperatures above 11° C. Neither can it be considered as a quiescent period following sexual reproduction, as thought by Dehorne, for that type of reproduction did not occur at all in our cultures. But cyst-formation is certainly affected by low temperatures as stated by Beddard, and this phenomenon takes place only when sufficient food is present so that reserves can be accumulated.

There exist several groups of fresh-water animals which hibernate during the course of their biological cycles; for example, the Bryozoa survive the winter period as statoblasts or hibernacula (Brien, 1936) and the sponges of the Spongillidae family, as gemmules (Brien, 1932; Leveaux, 1939). In both instances, the organisms could not otherwise withstand the winter cold. They form buds which consist of a mass of undifferentiated cells containing large quantities of nutritive reserves and surrounded by a protective covering. In the following spring, these cells multiply and differentiate, the shell splits open and a new individual escapes.

The same cannot be said of Aeolosoma. Here, the whole animal is enclosed within the cyst and its life processes are slowed down. Transformations occurring in the reserve tissues are not as pronounced as in Bryozoa or sponges.

In natural surroundings, cyst-formation may be explained in the following manner: in autumn, the water becomes colder and richer in products of decomposition from vegetable matter: Aeolosoma then stores its reserves. When the water is sufficiently cold, the worm becomes encysted and falls to the bottom of the creek into some hollow spot which does not freeze. In this way the worm can resist a temperature of a few degrees above 0° C. during winter. When the water warms up in spring, the worm emerges and asexual reproduction is resumed. The biological cycle can thus take place without the occurrence of sexual reproduction.

However, this last type of reproduction was observed in a few cases in *Aeolosoma hemprichi* and *Aeolosoma quaternarum* but it did not occur in any of our cultures though submitted to various environmental conditions. Here, as in other limicolous Oligochaeta, for example *Chaetogaster diaphanus*, where asexual reproduction is very intense, sexuality is a secondary phenomenon which appears only in certain definite circumstances. Scissiparity alone can definitively assure the survival of the species, and in places where the winter months are rather cold, the individuals survive by encystment.

SUMMARY

(1). The fresh-water oligochaete, *Aeolosoma hemprichi*, hibernates in the form of a cyst.

(2). When the worms are placed in a rich nutritive medium at 6° C., their movements are slowed down, and their growth stops. At this period, chains are formed of two zooids, at the most. Meanwhile, the worm accumulates food reserves, becomes opaque and secretes a mucus which will harden and form a transparent cyst.

(3). The worm stays motionless inside the cyst during the entire cold period. When the temperature increases, it becomes active again, uses up its food reserve and becomes transparent. After about fifteen days, it emerges and resumes its growth according to certain definite laws of scissiparity particular to this species.

REFERENCES

BEDDARD, F. E., 1905. Oligochaeta. Oxford.

BEDDARD, F. E., 1905. Note upon certain species of Aeolosoma. *Ann. Mag. Nat. Hist.*, **6**: 262–265.

BEDDARD, F. E., 1889. Note upon the germ cells in the integument of Aeolosoma. *Proc. Zool. Soc.*, **16**: 51–56.

BRIEN, P., 1932. Regénération naturelle chez les Spongillidae. *Arch. Zool. Exp. Gen.*, **74**: 461–506.

BRIEN, P., 1936. Contribution à l'étude de la reproduction asexuée chez les Phylactolémates. *Mem. Mus. Hist. Nat. Bruxelles*, 569–626.

DEHORNE, L., 1916. Les Naidimorphes et leur reproduction asexuée. *Arch. Zool. Exp. Gen.*, **56**: 88.

HERLANT-MEEWIS, H., 1950. Les lois de la scissiparité chez *Aeolosoma hemprichi*. *Bull. Soc. Canad. Biol.*, **2**.

HERLANT-MEEWIS, H. AND BOULANGER, J. P., 1949. Le genre Aeolosoma. *Compte-rendu A.C.F.A.S.*

LEVEAUX, M., 1939. La formation des gemmules chez les Spongillidae. *Ann. Soc. R. Zool. Belgique*, **70**: 53–96.

MEEWIS, H., 1933. Remarques au sujet de la scissiparité chez les Oligochétes. *Ann. Soc. R. Zool. Belgique*, **63**: 117–140.

STEPHENSON, J., 1930. Oligochaeta. Oxford.

STOLC, A., 1889. On the structure of Aeolosoma. (In Bohemian.) *Sitz. Ber. Bohm. Ges.*, 183–194.

VEJDOWSKY, F., 1884. System und Morphologie der Oligochaeten. Prague.

THE RELATION BETWEEN THE SCATTERING LAYER AND THE EUPHAUSIACEA[1]

HILARY B. MOORE

University of Miami Marine Laboratory, Coral Gables, Florida

Introduction

During and since the war, workers in various laboratories have reported that in the process of underwater sound experiments, mid-water echoes have been obtained from what has come to be referred to as the "Deep Scattering Layer." Results of observations made by the University of California have appeared in a series of reports (Univ. Cal., 1942–1946), while others from the Woods Hole Oceanographic Institution have been published by Hersey and Moore (1948), and Moore (1948). Papers by Raitt (1948), Dietz (1948) and Johnson (1948) describe more recent work on the subject. Most of the work which has so far been done on the scattering layer has been of a physical nature, and it seems most desirable at this stage that the problem should be discussed from a biological aspect.

It is a difficult matter to prove directly what material is responsible for an echo in the sea. The alternative approach is to determine what materials present in the sea are capable of producing echoes of the observed type and then to determine which of these exhibit identical characteristics of distribution, diurnal migration, etc., with the echoes. These characteristics are of so complex a type that, if an organism can be found which parallels them, the probability becomes extremely high that it is responsible for the observed echo phenomena. It must be realized that throughout this paper we are endeavoring to make such a comparison: available data on the scattering layer and on certain zooplankton are compared, and at the end an estimate of the probability of the correctness of the solution, rather than a proof, is presented.

This paper is presented in three sections: the first deals mainly with the scattering layer, the second with the biology of euphausids and the appendix with the computation of illumination. Since work on all lines has developed concurrently, it is not possible to separate them completely, and in many cases it is necessary to interpret observations made in one section in terms of observations in the other section.

The Scattering Layers

General. Most of the data discussed here have been obtained from bathygrams.[2] Although oscilloscope records of individual signals afford much greater detail, the continuous spark-recordings have the great advantage of allowing the complete

---

[1] Contribution No. 535 from Woods Hole Oceanographic Institution. Contribution No. 47 from University of Miami Marine Laboratory.

[2] There appears to be need for a term descriptive of records from echo sounders, and not suggestive, by implication, of a particular trade product. For this reason we propose the word "Bathygram," and use it throughout the paper.

picture for a twenty-four hour period to be examined as a whole. The scattering layer usually appears on these bathygrams as a darkened strip, lying, in the daytime, at a depth of two to three hundred fathoms. Between sunset and sunrise it approaches the surface, and usually is masked there by the record of the outgoing signal (Fig. 1). Most often there is only a single layer, but on a number of occasions two or more distinct layers have been recorded, and there is little doubt that improved techniques, including, especially, shortening of the ping length, will allow the separation of such multiple layers more regularly in the future. There may also be layers which do not execute the same type of diurnal migration as the main layer. Finally there is often a very deep layer lying at a depth of about five hundred fathoms, and this also may execute a diurnal migration. Most of the bathygrams shown here have been horizontally condensed by a photographic method described by Moore (1949c) which gives four-times horizontal reduction while retaining the vertical dimensions unchanged. In addition to allowing better presentation of the records, this method reduces the curvature of the vertical coordinates to one-fourth, thus reducing the skewing normally present in the upper parts of the bathygrams. The methods used for correcting the recorded depth of the scattering layer for the effects of depth and ping length have been referred to in Hersey and Moore (1948).

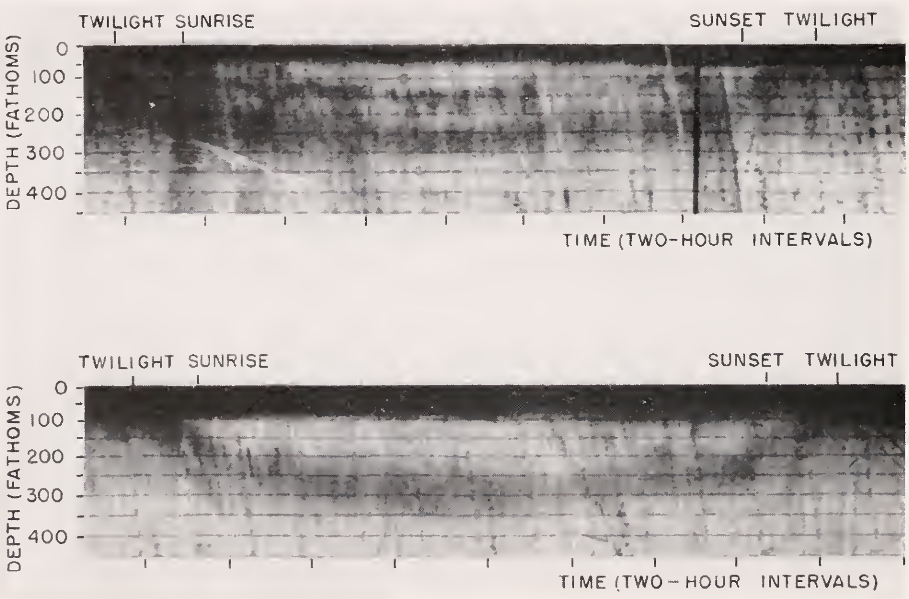


FIGURE 1. Condensed bathygrams showing double and single layers. Depth intervals, 50 fathoms. A very deep layer can be seen at sunrise and sunset in the lower figure. a. *Atlantis* cruise 152, 14 Aug., 1948; 42°44′N., 27°51′W. b. *Atlantis* cruise 152, 17 Aug., 1948; 39°15′N., 26°39′W.

Possible sources of the scattering layer effect. There is no known physical agency which could produce an echo of the scattering layer type, with its marked diurnal vertical migration. It appears certain, therefore, that we must be dealing with a living form. The phytoplankton may be ruled out both because it does not migrate vertically and because its very small size makes it a less probable source than some of the larger forms. Sargasso weed is concentrated close to the surface, and any found at two hundred fathoms will almost certainly be sinking steadily. Further, it is absent from many parts of the ocean from which the scattering layer has been recorded. Of the animal population, we may consider separately the smaller zooplankton, and the larger and more active forms such as fish and squid. Fish, many of which have swim-bladders containing gas, offer an attractive explanation, and we know so little of their abundance in the open ocean and of their diurnal migrations, that it is difficult to evaluate their significance. However, there are certain arguments against their being the source of the scattering layer. Firstly, being predators, they must be present in much smaller total quantity than the zooplankton on which they feed. Secondly, being considerably larger than the zooplankton, they have, relatively, a much smaller surface area. Dietz (1948) also points out that many of the commoner oceanic fish which are known to inhabit the depths in question, do not have swim-bladders. Finally, all the records of assured fish shoals which we have seen have been of a very patchy and discontinuous nature, quite unlike the extremely uniform records from the organisms composing the scattering layer.

The above arguments may also be applied to Lyman's (1948) suggestion that squid may be the source of the scattering layer. Further, the geographical distribution of odontocete whales, and hence presumably that of the squid on which they feed, is not in the least like the distribution of the scattering layer in those areas of the north Atlantic which we have studied. Unfortunately the paper by Townsend (1935), on the distribution of the squid-eating sperm whales, does not include comparable data on the common north Atlantic euphausid-eating whales, so comparison of the distributions of the two types of whale with that found for the scattering layer, is not possible.

Of the zooplankton, certain groups inherently appear to be unlikely sources of the scattering layer. Subject to the results of experimental tests now in progress, it would appear less probable that an echo would be returned from an animal with a gelatinous body such as a medusa, siphonophore, or chaetognath, than from one with a more solid musculature and a chitinous or calcareous surface. Furthermore, data obtained in the Bermuda area (Moore, 1949a), do not indicate any particular concentration of these gelatinous forms at the depth of the scattering layer, the greatest numbers being considerably nearer the surface. The same is true of the forms such as pteropods and some heteropods which have a calcareous shell; in addition, most of these show a marked seasonal variation in abundance, which variation has not been observed for the scattering layer.

There remain the crustacea, which have a chitinized surface; of those crustacea which are sufficiently abundant and widely distributed to justify consideration, there are three main groups. The large red prawns (*Acanthephyra*, *Sergestes*, etc.) and some euphausids concentrate in the daytime at a depth of five to eight hundred fathoms (Waterman *et al.*, 1939; Leavitt, 1935, 1938) and show a definite diurnal vertical migration. These are too deep to be the source of the regular scattering layer, but they might account for the very deep layer to which we have referred. The euphausids, usually one to two centimetres long, agree well in their distribution and behaviour with the scattering layer, and it is with these that most of the present paper is concerned. The copepods, usually only a few millimetres long, have their main concentration too shallow to be the source of the scattering layer, but they may well be concerned in reverberation. Raitt (1948) has made calculations with regard to the effective size of the organisms concerned, but we cannot yet interpret this in terms of actual physical size.

Quantitative estimate of euphausid abundance. It is necessary that the organism proposed as the source of the scattering effect be present in adequate numbers to produce the observed effect. We do not yet know the reflective properties of euphausids, although it is hoped that work now in progress will provide data on this. We are able to give some figures on the abundance of euphausids which may prove useful in future calculations. A series of net hauls was made near Bermuda at a time of night when most of the euphausids were concentrated near the surface. Oblique hauls were made at various depths from the surface down to approximately 110 fathoms, the main concentration of euphausids being at about 40 fathoms. The average catch for all depths was 273 euphausids per haul of a 70 cm. diameter net, towed for thirty minutes at 2 knots. Assuming a filtration efficiency of 50 per cent for the net, this indicates 130 euphausids in a vertical column of water one square metre in section and 200 metres deep. Those individuals below the depths sampled would probably bring this figure for the total population up to 150. The greatest concentration observed corresponded to about 1.5 euphausids per cubic metre at night, and about half this figure in the daytime.

Calculations from data of Leavitt's (1938) indicate a maximum concentration of 1.4 per cubic metre, making no allowance for a filtration factor, or 2.8 including the factor. He used larger nets (2 m. diameter), so the escape factor referred to below would probably be less in his case.

It is well known that a plankton net is inefficient in capturing the larger and more active forms, and that a considerable proportion of them are able to avoid the net. While we have no exact data on this, we have the results of night surface net hauls made at Bermuda simultaneously with visual estimates of the abundance of the brightly luminescent euphausids. From these it would appear that a net indication of only one euphausid per 75 cubic metres of water corresponded with a visual estimate of at least one per cubic metre. It would seem legitimate, therefore, to multiply our net estimates by at least ten, and probably more. This would give a value of 1500 euphausids in a column of water of one square metre cross section, extending from the surface to below the scattering layer, and maximum concentrations of 15 per cubic metre at night, 7 in the daytime. Assuming a cross section of 0.5 square centimetres for an average euphausid, when viewed from above this would mean that a vertical beam of sound would suffer at least ten per cent interception by euphausids by the time it had passed through the scattering layer, and this figure is probably too low. Finally, our estimate was made in the Bermuda area where the standing crop of plankton is comparatively poor. Nearer the continental shelf, where much stronger scattering layer records have been obtained, the zooplankton is known to be considerably richer.

Correlation between the geographical distribution of the intensity of scattering layer and that of euphausids. From our own North Atlantic and Mediterranean records, and from those of Dietz and others for the Pacific, it appears that the scattering layer is of extremely widespread, if not universal occurrence in deep water. There are not, at present, adequate data on either the quantitative geographical distribution of euphausids, or the intensity of the return from the scattering layer, to allow an overall correlation between the two. However, for the Mediterranean we have some figures which can be used (Moore, 1949b). The nutrient salts in this area decrease from west to east (Helge, 1931; Bumpus, 1948). Jespersen's (1935) figures for total zooplankton decrease similarly (Fig. 2a). Data of Ruud's (1936), which are not well suited for such a quantitative comparison, nevertheless give some indication of a similar decrease in the euphausid population (Fig. 2b). For those parts of the Mediterranean which were examined by the *Atlantis*, scattering layers were found mainly in the western area, with a few in one small area of the eastern Mediterranean (Fig. 2c). Oscilloscope records gave some support to the belief that, where the echo sounder failed to detect a scattering layer in this area, the layer was in fact weak or absent. In this area, then, there is agreement between the distribution of the scattering layer and of the zooplankton in general and euphausids in particular. We have also recorded a sharp break in the intensity of the scattering layer record at the transition point between gulf stream and northern water.

Correlation between the geographical variations in depth of the scattering layer and of the euphausids. If euphausids are responsible for the scattering effect, there should be a correlation between the day level of the layer throughout the ocean and the proportions of relatively deep- or shallow-living species forming the local population. Such a comparison is possible along a line from Bermuda to Florida and further west into the northern part of the Gulf of Mexico. The source of the biological data is given in a later section. We are probably correct in arranging the commoner euphausid species of the area in the following order of increasing average depth: *Euphausia tenera*, (*E. americana* ?), *E. brevis*, *E. hemigibba*, *Thysanopoda aequalis*, *Thysanoessa gregaria* (Fig. 19).

Figure 3 shows the area of dominance of the various species along the line in question. Their distribution suggests that, north of Bermuda, where *E. tenera* and *E. brevis* are common, the layer should be fairly shallow. Further west the deeper-living of these, *E. brevis*, becomes more abundant, and then in turn mixes with and is replaced by the still deeper *E. hemigibba* and *T. aequalis*. Around Florida *E. americana* becomes dominant, and we believe that it is a shallow form. In the Gulf of Mexico, the shallow *E. tenera* is the common form. Finally, the abundance of the usually very deep *T. gregaria* to the south of Bermuda, should mean that the layer will be particularly deep there. Figure 4, which gives the noon level of the scattering layer throughout this area, agrees well with this. The layer is shallower north of Bermuda, and much deeper towards the south. Westwards it becomes first deeper and then shallower again, and in the Gulf of Mexico some of the observations are extremely shallow. Naturally, with observations taken at different times, and over so long a period, no exact agreement can be expected, but what there is would appear to be significant.

Another test of geographical distribution is afforded by double layering. In most

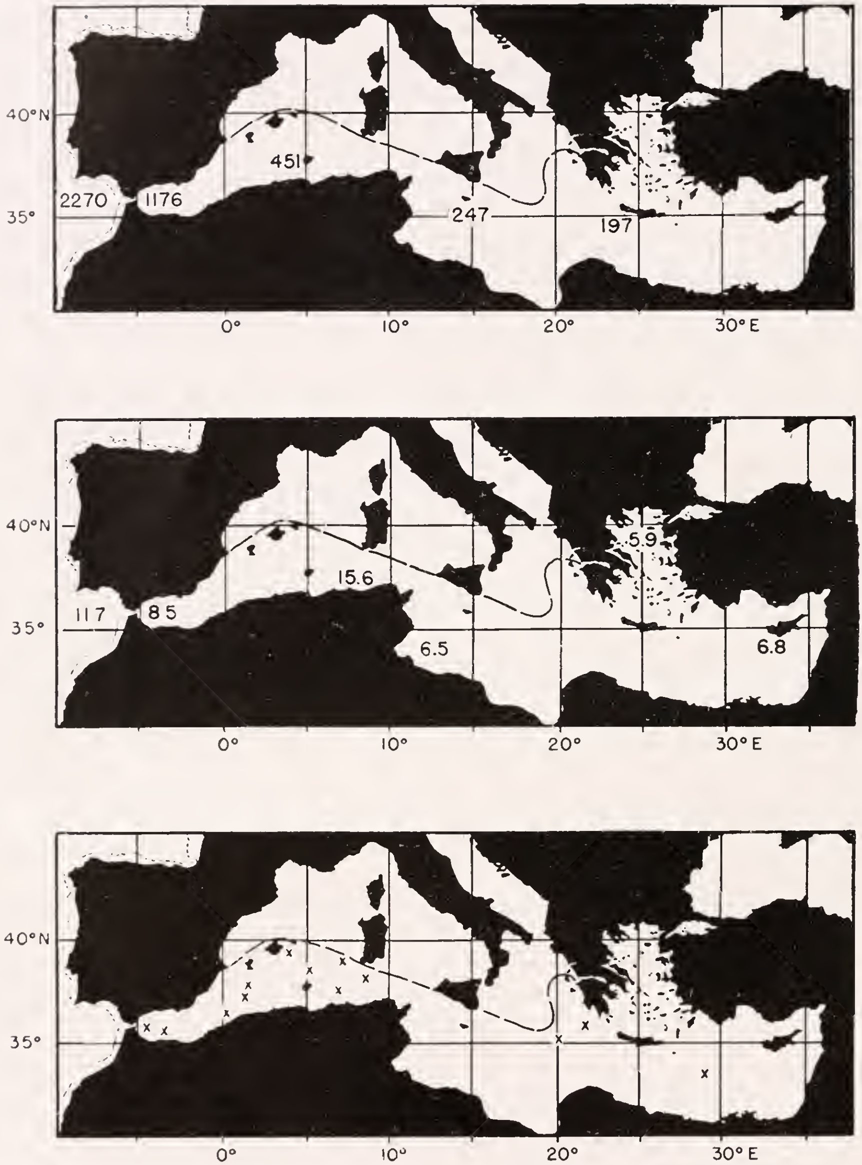

40°N
35°
2270
1176
451
247
197
0°
10°
20°
30°E
40°N
35°
117
8 5
15.6
5.9
6.5
6.8
0°
10°
20°
30°E
40°N
35°
0°
10°
20°
30°E

FIGURE 2. Comparison of plankton distribution and the occurrence of scattering layer returns in the southern Mediterranean Sea. a. Average volume (cc.) of macroplankton in the upper water layers (25–65 m. wire). (After Jesperson, 1935.) b. Relative numbers of euphausids per net haul in different areas of the Mediterranean. (Mod. after Ruud, 1936.) c. Distribution of scattering layer returns in the Mediterranean Sea. (No records were made north of the broken line.)

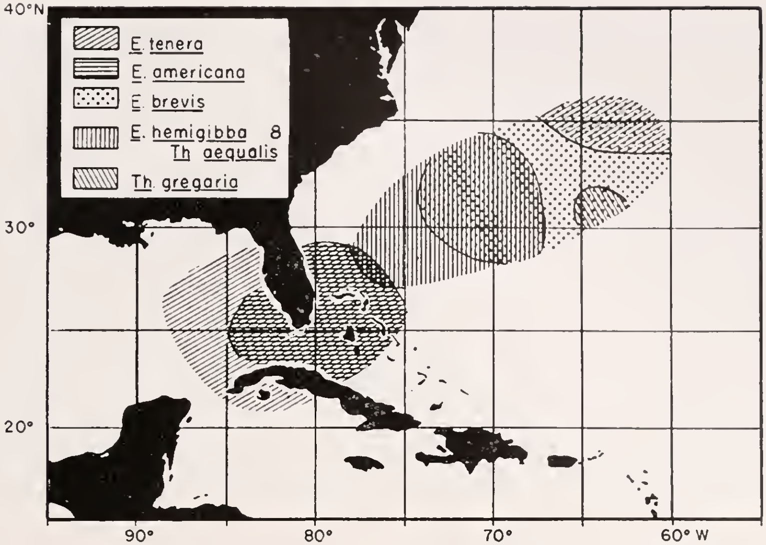

40°N
30°
20°
90°
80°
70°
60° W
E. tenera
E. americana
E. brevis
E. hemigibba & Th aequalis
Th. gregaria

FIGURE 3. Areas of dominance of euphausid species in Western Atlantic. (Information is lacking in unshaded areas.)

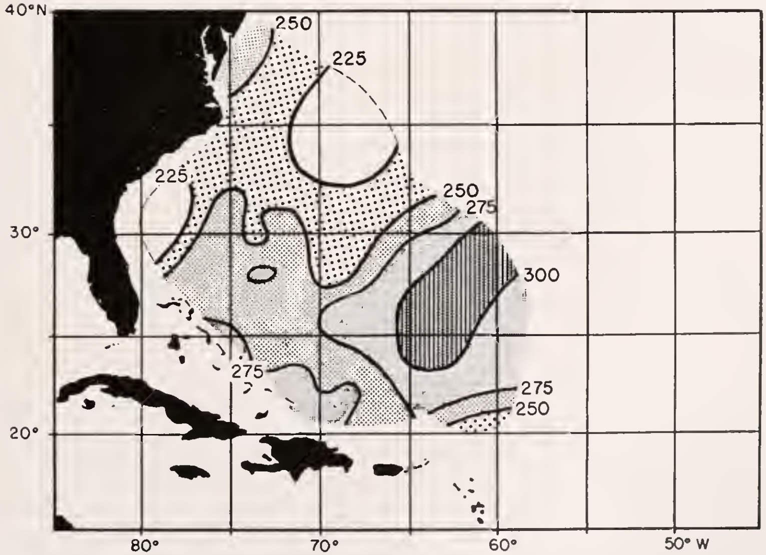

40°N
30°
20°
80°
70°
60°
50° W
250
225
225
250
275
300
275
275
250

FIGURE 4. Geographical trend of the noon depth of the scattering layer. (Figures represent the corrected approximate depth in fathoms of the peak abundance of scatterers.)

areas there are several species of euphausid present, each with its particular maximum level, but also with a fairly wide vertical spread. The long ping length used tends to reduce the detail readable from the bathygram. It is not surprising, therefore, that separation into distinct layers is rather rarely seen on the present records.

There is an area to the north of the Azores where the euphausid population consists almost entirely of two species, *Euphausia krohnii* and *Nematoscelis megalops*, which appear to have markedly different vertical distributions. They occur in approximately equal numbers at some localities, while at others *N. megalops* is the commoner. South of the Azores these species are scarce or absent and the populations are mixtures of several species with less distinct vertical distributions. Figure 5 shows the data available on the euphausids of this area, and Figure 6 the locations at which very marked separation into double layers was recorded. That the separation of the two layers in the records is in fact the result of the dominance of these two species is confirmed by the fact that at one station in the western Mediterranean where the same two species were dominant, similar double layering was recorded. Examples of a single and a double layer fathogram from this area are shown in Figure 1. Dietz (1948, p. 436) shows a clear example of double layering from the north Pacific.

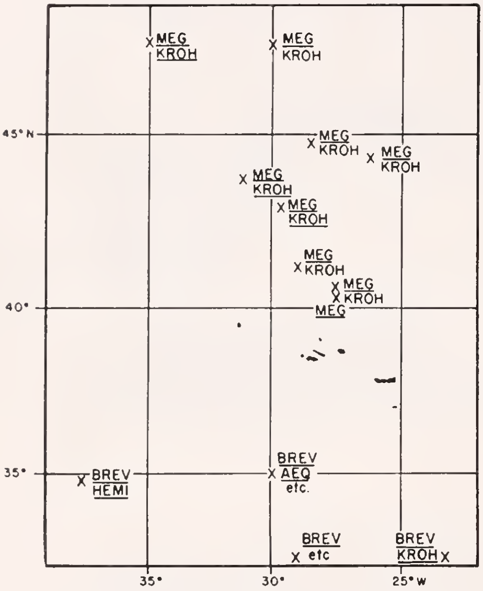


FIGURE 5. Dominant euphausids of the Azores area (most abundant species are underlined).

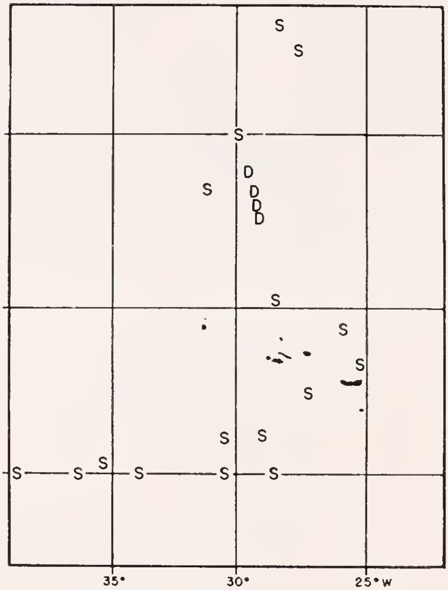


FIGURE 6. Occurrences of double and single echo in the Azores area.

Occurrence of the scattering layer in relation to the depth of the bottom. The oceanic euphausids are adapted to live permanently in mid-water, and do not normally ever touch the bottom. Net hauls show them to be absent from shallow inshore waters. Figure 7 shows the disappearance of the scattering layer at a point where the depth of water is slightly greater than that of the center of concentration of the layer, and similar records have been obtained where the water shallows on the edge of the continental shelf. In some inshore waters there are known to be

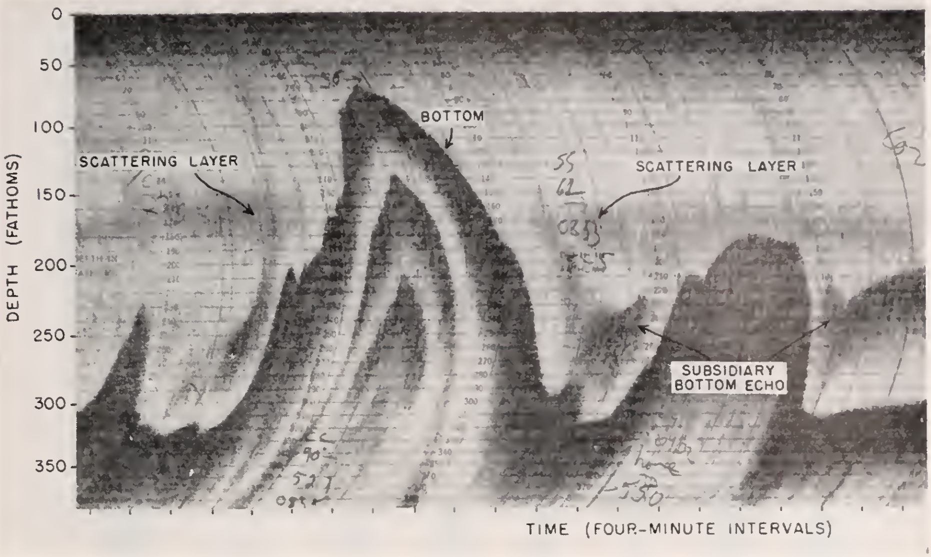


Figure 7. Bathygram showing scattering layer (160 to 190 fathoms) interrupted by submarine peak. *Atlantis* cruise 156, 4 June, 1949; 38°11′N., 73°30.5′W.

euphausids such as *Meganyctiphanes norvegica, Nyctiphanes couchi*, etc. which lie on, or close to, the bottom in the daytime. There are also Mysids with similar habits which may be very abundant in some inshore waters. Figure 8, which was made in water 100 fathoms deep near Miami, Florida, apparently shows such a form leaving the bottom about 40 minutes before sunset and reaching the surface just after sunset. It also shows clearly the phenomenon of downward diffusion after dark, and it should be noted that there was no moon on that occasion. Figure 9 shows a morning descent onto the bottom which, in this case, is at 200–250 fathoms.

Diurnal migration of the scattering layer and of plankton. The diurnal migration of the scattering layer is one of its most striking characteristics, and one which makes it practically certain that the source of the effect is an animal population. Discus-

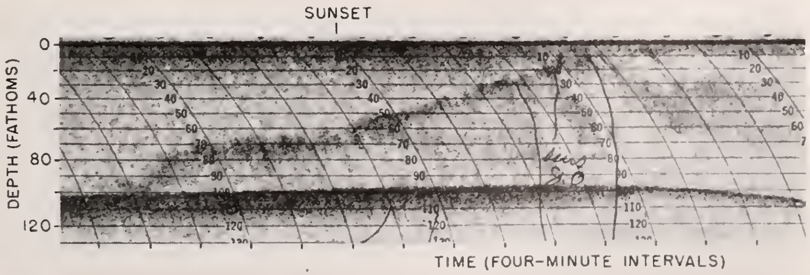


Figure 8. Bathygram showing evening ascent of layer from the bottom. *Atlantis* cruise 144, 11 April, 1947; 25°16′N., 80°05′W. (off Miami, Florida).

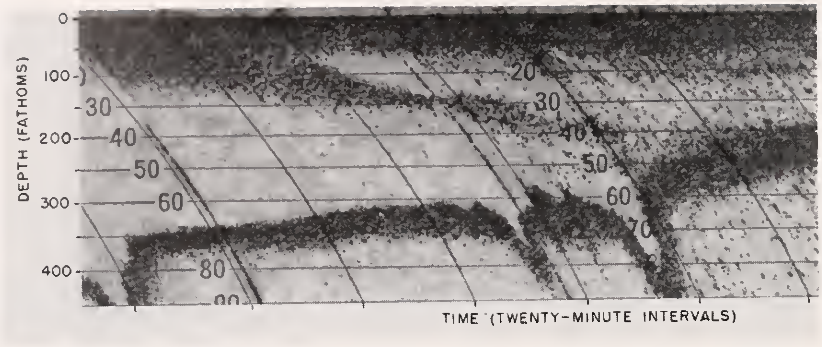


FIGURE 9. Bathygram showing morning descent of scattering layer onto bottom at 200 to 250 fathoms. *Atlantis* cruise 154, 7 Apr., 1949; 25°51′N., 79°41½′W.

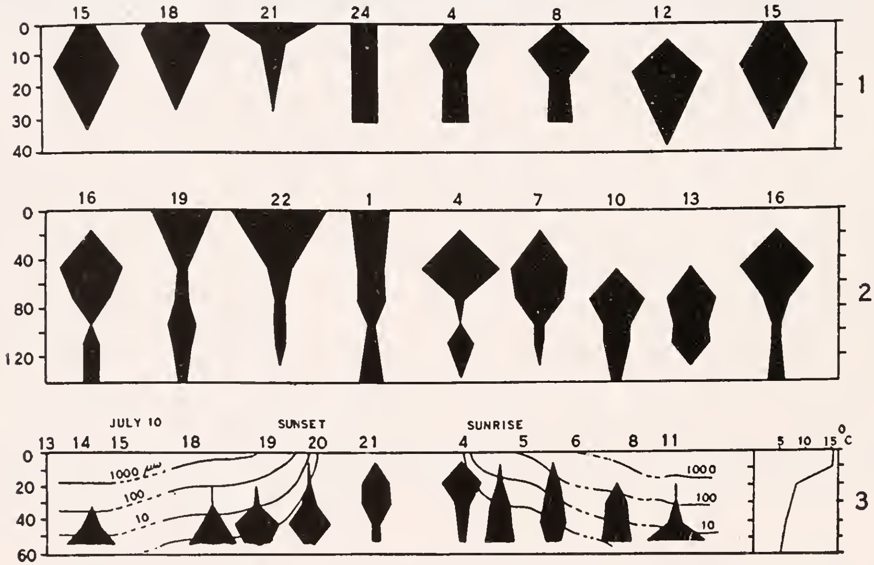


FIGURE 10. Vertical distribution of plankton animals. (From Russell, 1931.)

1. Hypothetical vertical distributions at different times in the twenty-four hours to illustrate the behaviour of a population of a species such as *Calanus finmarchicus* in its diurnal movements (after Russell, 1927, p. 237, Fig. 4).
2. Diagram showing vertical distribution of female *Calanus finmarchicus* at 3-hourly intervals during 24 hours on January 25th–26th, 1932; sunset 4.27 p.m.: sunrise 8.57 a.m. (after Nicholls, 1933, p. 150, Fig. 4).
3. Diurnal migration of adult females of *Metridia lucens* in the Gulf of Maine, July, 1932. The changes in light intensity are indicated by the lines representing the depth at which 1000, 100 microwatts etc. occurred (one microwatt may be taken as approximately equal to three metre-candles). The temperature curve at the right shows that migration right to the surface at night was prevented by the thermocline (after Clarke, 1933, p. 426, Fig. 5).

sion of some deductions made from bathygrams is deferred to the later section on the biology of euphausids, but some of them require consideration here.

Russell (1931) has presented a hypothetical pattern of diurnal variation in vertical distribution for a copepod, *Calanus finmarchicus* (Fig. 10). This shows a sequence of upward movement around sunset, followed by a general diffusion during the dark period; some upward concentration again immediately before sunrise, and descent to the day level when the sun rises. He also gives a graph of actual distributions observed by Nicholls (1933) for the same copepod which very closely parallel his own hypothetical figures. The figure of Clarke's (1933) for the same species, which Russell gives, has iso-illumination curves superimposed, and it is clear that the migrations tend to follow the iso-illumination curves. In Clarke's observations, however, the warm water at the thermocline appears to form a barrier which limits upward migration. A figure showing the diurnal migration of euphausids is given in Hersey and Moore (1948).

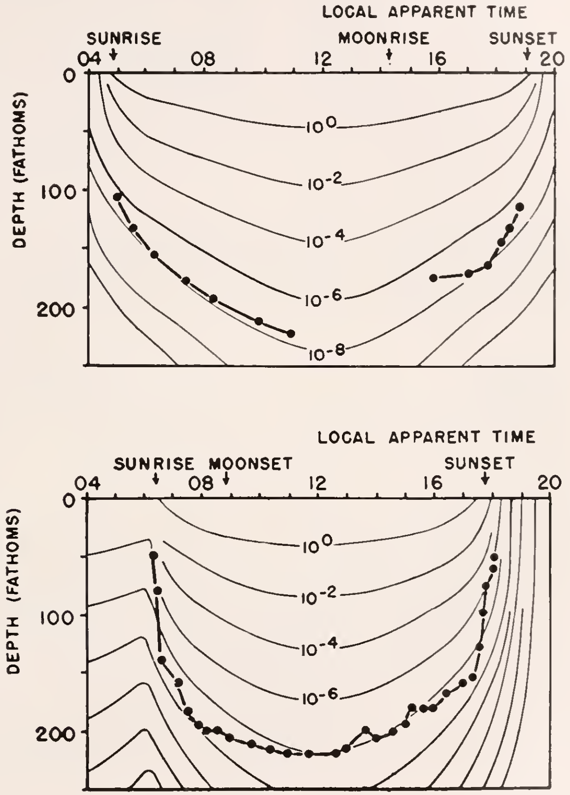


FIGURE 11. Diurnal movement of scattering layer and of iso-illumination curves. a. *Atlantis* cruise 151, 18 May, 1948; 38°51′N., 03°27′E. b. *Atlantis* cruise 144, 8 Feb., 1947; 25°07′N., 92°35′W.

Illumination is the chief factor motivating the upward and downward migrations, but, as shown later, limiting temperatures may form barriers at the two ends of the migration. Figure 11 shows records of the migrations of the scattering layer, taken from bathygrams, which closely parallel the curves of iso-illumination. Figure 12 shows that the layer may migrate in response to moonlight as well as to sunlight. Dietz (1948) has shown that the scattering layer was lost when he entered Antarctic waters during the period of continuous daylight, though its reappearance showed

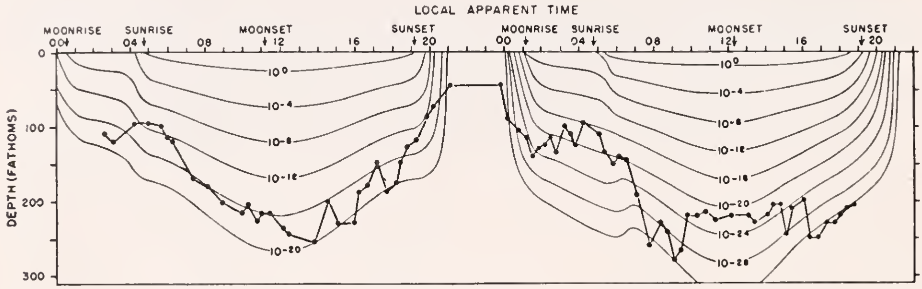


FIGURE 12. Diurnal migration of scattering layer showing, on second day, two periods of descent, one at moonrise and the other at sunrise. Deepening of layer in latter half of second day was associated with an increase in water temperature. *Atlantis* cruise 141, 23–24 May, 1946; noon locations: 38°26′N., 74°59′W. and 36°17′N., 74°41′W., respectively.

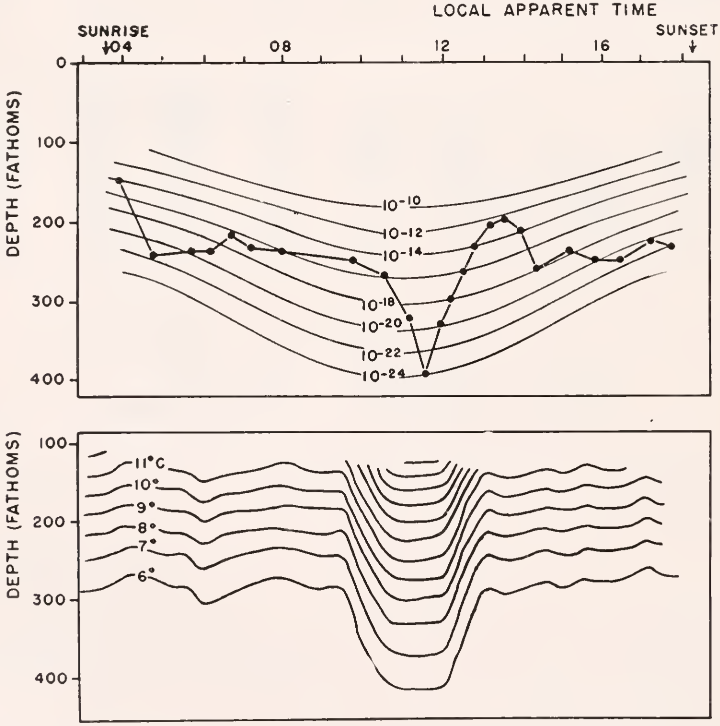


FIGURE 13. Diurnal migration of scattering layer showing its relation to temperature and illumination. *Atlantis* cruise 149, 1 July, 1947; 36°59′N., 66°57′W. a. Scattering layer and iso-illumination curves. b. Isotherms.

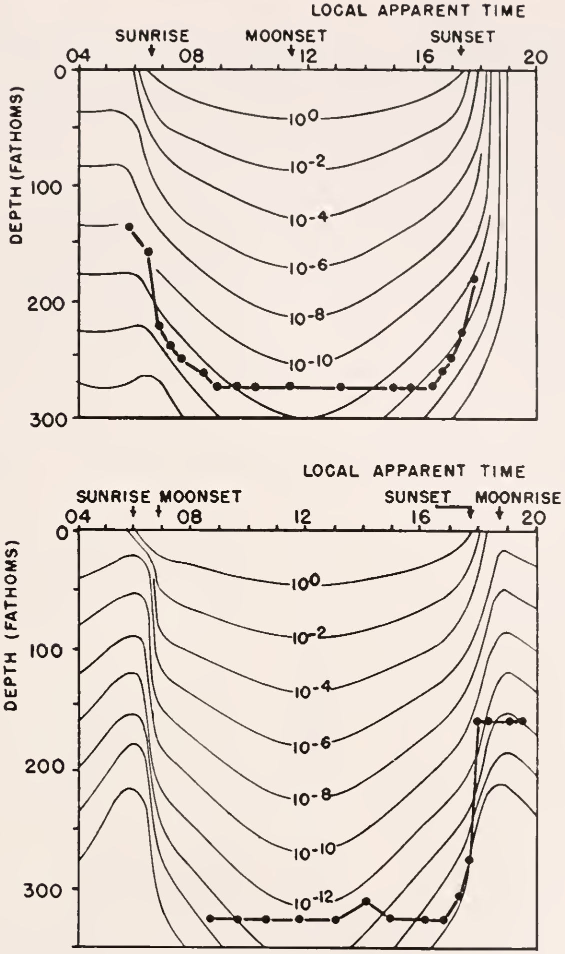


FIGURE 14. Diurnal migration of scattering layer showing marked noon flattening. a. *Atlantis* cruise 144, 13 Jan., 1947; noon position: 21°21′N., 75°39′W. b. *Atlantis* cruise 143, 11 Oct., 1946; noon position: 26°04′N., 60°55′W.

only approximate agreement with the return of the ship to an area of alternating day and night. He compares this effect with Bogorov's observations (1946) on the cessation of diurnal migration of the plankton in the Arctic during the summer period of permanent daylight. The disappearance of the scattering layer suggests, however, that in addition to ceasing to migrate, the organism responsible for the scattering effect becomes too diffuse vertically to show as a distinct layer in the bathygrams.

Relation of the depth of the scattering layer to temperature. Since the specific constitution of the zooplankton varies geographically, and different species have different optima and limits for temperature and other factors, no simple, overall correlation between the depth of the scattering layer and temperature can be expected. In a limited area where the plankton constitution is known to be more or less constant, such a correlation might be sought. Figure 13 shows a record of the variation in depth of the layer on a day when the *Atlantis* passed abruptly into much warmer water and back (the edge of the Gulf Stream). The coincidence of the sudden deepening of the layer and the warmer water is clear. The record was made on the edge of an eddy of cold water trapped on the Sargasso Sea side of the Gulf Stream. In another case, the coincidence of this change in depth of the scattering layer with the observed temperature changes in the water was so good that it was possible to make use of it in mapping the warm-cold interface. Unfortunately we cannot be sure, in these cases, that we were dealing with a similar zooplankton community in the cold and warm masses of water. In the case of most bathygrams from the open ocean we can be fairly certain that we were dealing with a similar zooplankton population throughout any one day.

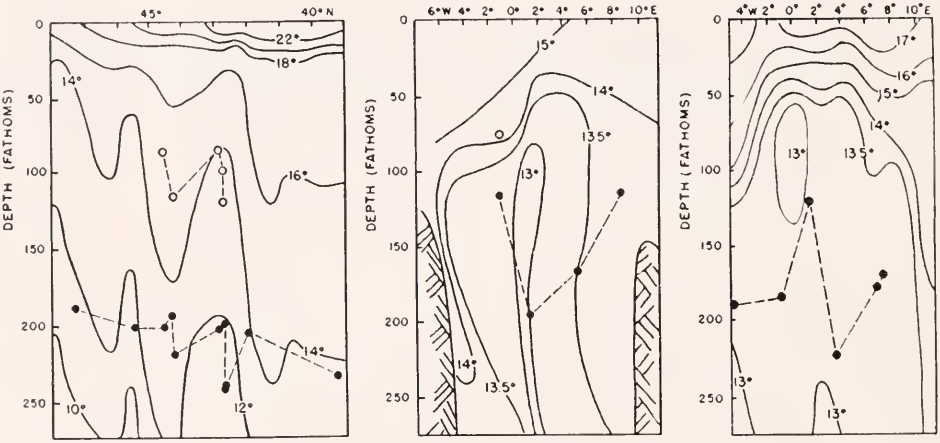


FIGURE 15. Noon scattering layer depths and temperature profiles. (Azore temperatures modified from Iselin, 1936; Western Mediterranean temperatures from Bumpus, 1948.) a. Azores area (July–Aug.). b. Western Mediterranean (Jan.). c. Western Mediterranean (Apr.–May).

The evidence so far presented shows a rather widespread similarity between the distribution and behavior of the scattering layer and those of euphausids. Assuming, for the moment, that the two are connected, we may examine the effects of temperature on their diurnal migration. Figure 14 shows two bathygrams in which the marked mid-day flattening of the scattering layer is interpreted as meaning that the euphausids met, at that depth, a cold barrier which interrupted their descent. We have already referred to the area in the neighborhood of the Azores in which the two observed scattering layers are believed to be produced by the two dominant euphausids *Nematoscelis megalops* and *Euphausia krohnii*. Figure 15a shows the noon depths of these two layers on a transect through the area, together with the temperature profile.[3] The more complete lower, or *Nematoscelis* layer shows an increase in depth paralleling the increase in temperature southwards. The two graphs shown in Fig. 15b and c were obtained from data from the area in the western Mediterranean where, again, a *Nematoscelis* layer could be distinguished. While the Azores series showed considerable noon flattening, which we interpreted as indicating the presence of a cold barrier to descent, the Mediterranean series showed little or none. It will be seen that in the Mediterranean, the *Nematoscelis* are living, on the whole, in warmer water than in the Azores area, and that they are in a zone with almost no thermal stratification. The generally shallower depths of the layer in the Mediterranean are in agreement with the higher extinction coefficients and lower illuminations relative to the Azores area. The fact that, in these areas where we believe that we are dealing with a single species of euphausid only, the occurrence of noon-flattening is dependent on low temperatures and thermal stratification, together with the dependence of the depth at which this noon-flattening occurs on temperature, gives considerable support to our temperature-barrier theory.

---

[3] The scattering layer observations, and those on which the isotherms are based, were made on different cruises, so exact agreement cannot be expected.

The fact that such a relationship emerges in an area where we know that we are dealing with an unusually simple euphausid population is another argument in favor of euphausids being responsible for the scattering effect. The evidence would, of course, be stronger if we had tow nettings made in the layer at all these stations, but this was not possible. Where we have taken tow nettings in the layer in other areas, we have found the main concentration of euphausids to be at the depth indicated by the bathygrams (Hersey and Moore, 1948).

Other types of scattering layer. So far we have discussed only the daytime distribution of the main scattering layer. While we have some night records, more will be needed before any analysis can be made. We have referred briefly to two other types of scattering layer, the very deep one, and one which apparently shows a reversed diurnal migration. A few complete day records of the deep one have been

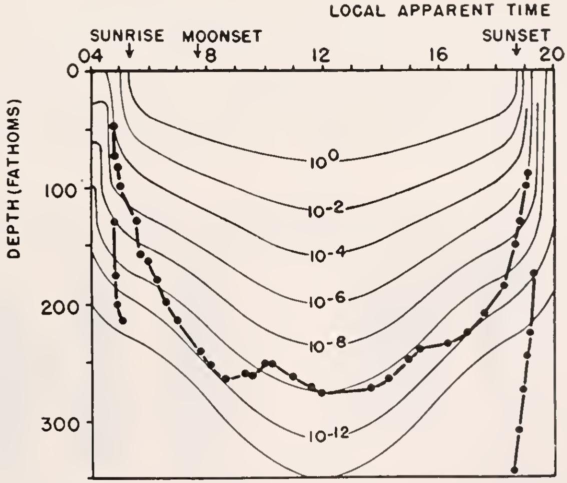


FIGURE 16. Diurnal migration of scattering layers, showing main layer throughout day and very deep layer around sunrise and sunset. *Atlantis* cruise 152, 22 Aug., 1948; noon position: 37°29′N., 27°18½′W.

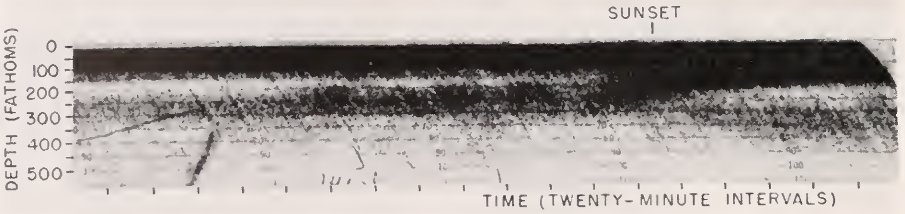


FIGURE 17. Bathygram showing evening ascent of one scattering layer and the simultaneous descent of another. *Atlantis* cruise 149, 7 July, 1947; noon position: 38°42'N., 69°32'W.

obtained, but more often the layer is indicated on the bathygram only around the periods of ascent and descent, and tends to be missed at its greatest depths (Figs. 1b, 16). It apparently performs a diurnal migration very similar to that of the main layer, but descends slightly earlier, and ascends slightly later. Its night level also appears to be deeper than that of the main layer. The day level is much deeper than that of the main euphausid concentration, and it is suggested that the organisms responsible may be the large Acanthephyrid and Sergestid prawns. Waterman *et al.* (1939), and Leavitt (1935, 1938) have shown that there is a concentration of these prawns and of certain euphausids at about the required depth. The prawns are considerably larger than the euphausids, but there is little information on their abundance. Being larger and more active than the euphausids, they are even less likely to be adequately sampled by nets, so it is quite possible that they occur in considerable numbers.

Bathygrams frequently show a record similar to that in Figure 17 in which, immediately after the evening ascent of the main layer, what looks like a continuation of this layer remains on at the same level or descends slightly. In Hersey and Moore (1948) an oscilloscope analysis of such a condition was given. The much greater detail possible with short ping length and oscilloscope recording, shows that there were, in fact, two layers, a deep one which ascended in the normal way at sunset, and a shallow one which descended and crossed it. The long ping length generally used on the echo sounder would obscure the details in such a crossing over and give a record similar to that shown in Figure 17. With only one series of oscillograms, it

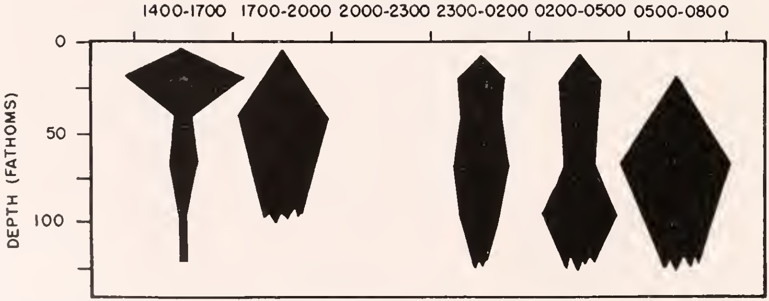


FIGURE 18. Diurnal migration of *Stylocheiron abbreviatum* and *S. carinatum* combined. Bermuda, 4–5 Feb., 1949.

cannot yet be said whether this is always the explanation of such records, but there is no apparent reason why it should not be.

Not much is known about plankton forms which show this reversed type of diurnal migration, although several workers have suggested the possibility. In Moore (1949b) it was shown that there was a correlation between the day level inhabited by various species of zooplankton and the extent of their diurnal migration. The greatest migration was shown by the deepest-living forms, while in shallow-living forms there was little or no migration, and in a few of the shallowest forms there appeared to be a downward movement at night. This statement is based on a number of day hauls but on only one series of night hauls, and further series are needed before we can be sure about the downward night migration. Among the euphausids, some of the species of the genus *Stylocheiron* appear to exhibit such a night descent (Fig. 18). The numbers taken were too few to prove the point, but it is tentatively suggested that some members of this genus and in particular *S. abbreviatum* and *S. carinatum,* might be responsible for the recorded night descent. It is, of course, quite possible that in this case some form other than a euphausid is responsible.

Euphausid Biology

Geographical and vertical distribution. Information on the oceanic distribution of planktonic forms, both vertical and geographical, is very incomplete, and unfortunately this is particularly true for euphausids. Einarsson (1945) gives much information for the northern part of the North Atlantic, but makes only brief reference to the more southern species considered here. Ruud (1936) gives considerable data on geographical distribution of euphausids in the Mediterranean, and a little on the relative vertical distributions of the species. Tattersall (1926) covered a good deal of the area between Bermuda and the American coast, and from about 23° to 37°N. Leavitt (1935, 1938) and Waterman *et al.* (1939) give good vertical distribution data at a few stations in the same area, and these, together with our own from near Bermuda (Moore, 1949a), are practically our only source of information on vertical distribution. The *Atlantis* has, for a number of years, taken night surface tow nettings throughout the North Atlantic, Mediterranean and Gulf of Mexico. Descriptions of these have not been published, but are made use of here. Information on diurnal migration is taken from Waterman *et al.* (1939) and from Moore (1949a).

For geographical distribution, we have considered all hauls in which an adequate number of euphausids was taken, and expressed each species as a percentage of the total euphausid population. This was necessitated for comparison of various types of hauls. In some cases either oblique hauls, or a series at different depths, yield a good sample of the population at all depths. Daytime hauls near the surface are likely to be poor in representatives of the deeper species. For the sake of conserving ship's time, we have made use largely of surface hauls taken around midnight. At that time the euphausids will have approached the surface, and tests at Bermuda showed that a fairly representative sample of all but the deepest forms was obtained. Since these night hauls form a large proportion of our data, and since we have reason to suspect that some species of the genus *Stylocheiron* descend at night, this whole genus has been omitted in calculating percentages. The combination of such mixed data is only justified by our need for information from a wide area, and the distribution patterns obtained must be considered significant as a whole rather than in detail.

From observations at Bermuda, which are summarized in Figure 19, and from the results of Leavitt (1935, 1938) and Waterman *et al.* (1939), we are probably correct in arranging the common species of the area in the following order of increasing depth of day level: *Euphausia tenera, E. brevis, E. hemigibba, Thysanopoda aequalis, Thysanoessa gregaria.* Ruud (1936) confirms the fact that *E. hemigibba* lives deeper than *E. brevis,* and that *T. gregaria,* though very variable, is a deep form. *E. americana* is believed to be a shallow form, comparable with *E. brevis* or *E. tenera.*

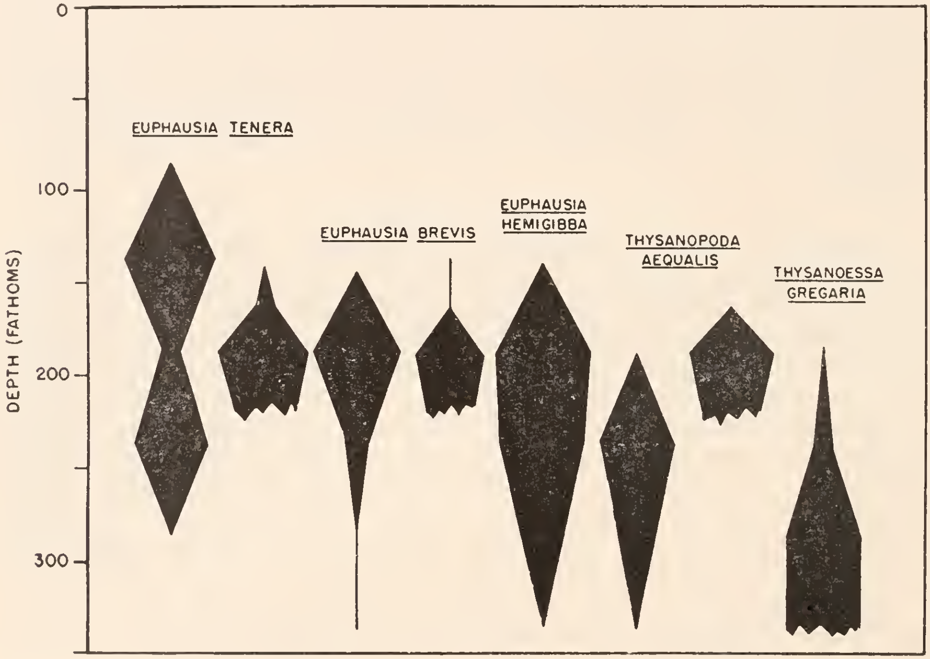


FIGURE 19. Vertical distribution of the commoner species of euphausids off Bermuda, from hauls made in the middle of the day.

E. krohnii also is probably comparable with *E. brevis,* and *Nematoscelis megalops* is deeper. *Stylocheiron* spp. rarely form a large proportion of the catches. Some of them are very shallow species, and so were taken in a sufficient number of Bermuda hauls to yield fairly accurate knowledge of their distribution. Of these, *S. suhmi* has a mean day level of 25 fathoms, *S. carinatum* of 44 fathoms, and *S. abbreviatum* of 57 fathoms.

Temperature and illumination—sources of data. Temperature data have been obtained from the nearest hydrographic station, supplemented where needed by the shallower but more numerous bathythermograph records. The latter were made at the same time as the scattering layer and net haul records, as were those at the hydrographic stations in the Mediterranean. In other areas older hydrographic information had to be used, but at the fairly stable depths with which we are concerned, this is not likely to give rise to much error except in areas of large fluctuation such as the margins of the Gulf Stream. The source of illumination data is discussed in the Appendix.

Diurnal migration and the environmental factors involved. There seems little doubt that illumination is the major factor in controlling diurnal migration, but the reaction of the animal to light is not simply one of moving so as to remain at a constant illumination. While the only known factors which ordinarily can produce a directional effect are light and gravity, the response of the animal to these can be modified by other factors such as temperature. Kikuchi (1930) has reviewed the factors which are so far known to affect diurnal migration. These may include internal factors as well as external factors so that different levels may be characteristic of males, females, and immature individuals of the same species. Russell (1931) points this out, and in another paper (1927) he refers to cases in which increased temperature may make a previously positively phototropic form negative, while cooling may have the reverse effect.

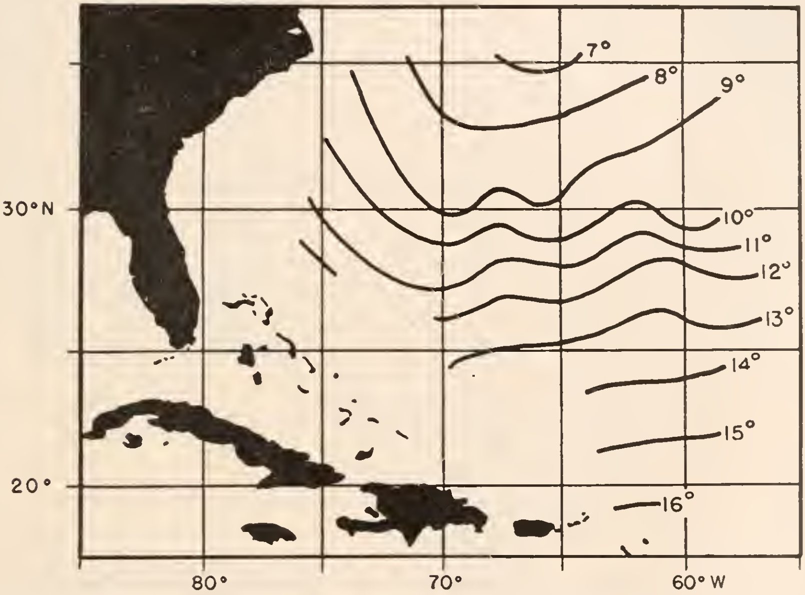


FIGURE 20. Difference in temperature (°C.) in summer between the surface and a depth of 328 fathoms.

The environment of animals living as deep as the euphausids differs in many ways from that of shallower forms, and some of the differences are not obvious at first sight. To begin with, in the course of their diurnal migration they will undergo considerable changes of temperature. Figure 20 indicates of what order these may be for euphausids in one section of the north Atlantic. Such changes must have very considerable effect on the physiology of the animals. They will also undergo a change in pressure of 50–60 atmospheres. Pressure is a factor which has received little consideration except in so far as it affects much deeper-living forms. Some results obtained by Hardy and Paton (1947) suggest that it may be operative, even at lesser depths. Reynard (1885) subjected various shallow-living forms to increasing pressure and found that they became at first more active, and then, with further increase, quiescent. From the summaries of Cattell (1935, 1936) it would appear that in general, and considering only comparatively low pressures, increased pressure on nerves increases their action potential and rate of propagation, and decreases their threshold of stimulation. In the case of striped muscles, above a critical temperature, increased pressure increases their contraction, while below that temperature it decreases it. Pressure changes of the order involved in a migration of three hundred fathoms might be expected to produce significant changes of this type.

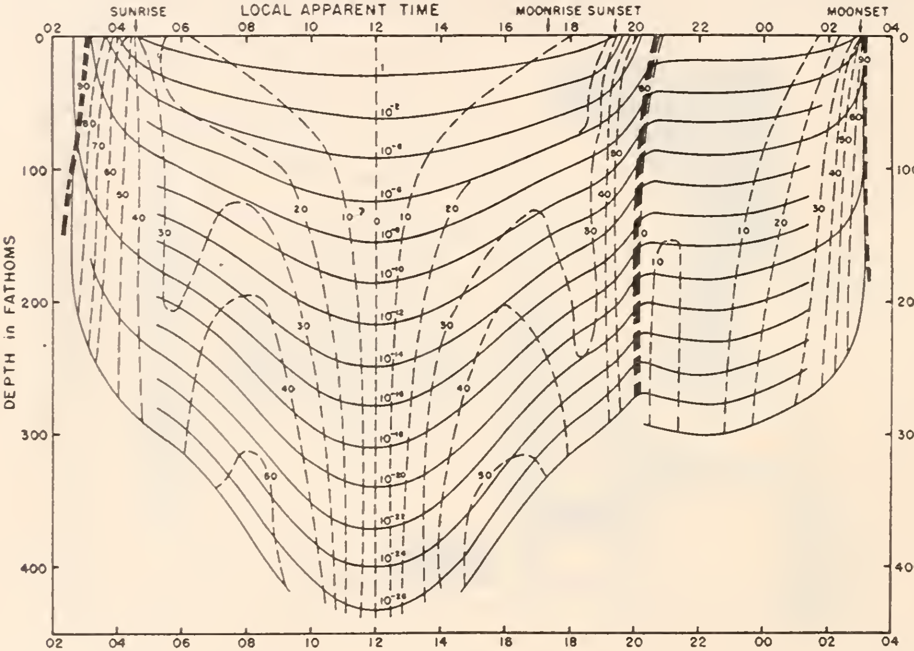


Figure 21. Hypothetical diagram illustrating diurnal changes in illumination at different depths. Iso-illumination curves (whole lines) show values related to a figure of 100 for overhead sunlight at the surface. Physiological stimulus (broken lines) is shown as curves of equal rate of change of illumination in arbitrary units.

Conditions with regard to illumination will be different in several ways from those for shallow-living forms. The level of illumination will be very much less, although Waterman *et al.* (1939) point out that there should be enough light to stimulate the eyes of crustacea at a depth of about 600 fathoms in the open ocean. If we refer to the iso-illumination curves (whole lines) in Figure 21 it is apparent that illumination changes are very different for shallow and deep forms. Assuming for the moment that the plankton migrates vertically so as to maintain itself always at its optimum illumination, then an animal whose day level is less than 100 fathoms will, in the example shown, be able to ascend right to the surface at night and still find less than optimum illumination, despite the fact that there is a nearly full moon. An animal whose day level is at 300 fathoms will find similar illumination during much of the night at about 150 fathoms, and would, at the surface, find illumination about 10^{13} times brighter than its optimum. Furthermore, in the northern hemisphere at full moon in winter, and when there is the maximum difference in declination between the sun and moon, the lunar rays will have so much shorter a path to travel in reaching a given depth than the solar rays, and their vertical absorption rate will therefore be so much less, that there should

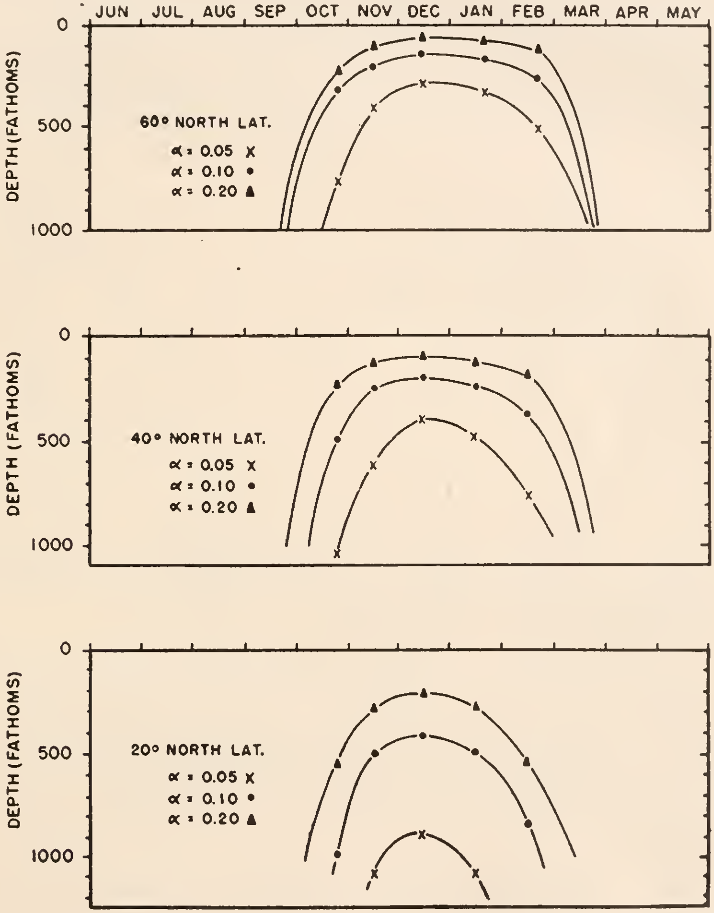


FIGURE 22. Relationship of depth at which illumination from full moon at transit equals that from noon sun with latitude, season, and extinction coefficient. (The depth is least at the moon's northernmost and the sun's southernmost transit.)

be a depth at which moonlight will be brighter than noon sunlight. It is realized that in making this statement no allowance has been made for the scattering of the light, and it may be that the scattering will mask this effect, but if our argument in the Appendix on the importance of scattering in oceanic water is valid, the point seems worth considering. Figure 22 shows the calculated depths at which full moonlight will equal noon sunlight, and the variation of these with season, latitude, and extinction coefficient. Even if scattering largely masks this effect, it is clear that for animals living in the daytime at a depth of a few hundred fathoms there is, at some times of year, much less difference between day and night than for surface forms. If the effect is genuine, and it would appear that it must be so at least in very high latitudes, then we would expect to find the curious situation of shallow forms migrating upwards at night because of decreasing illumination, while at the same time deeper forms were moving downwards at night because of increased illumination.

Figure 21 shows another difference in environment for deep and shallow forms. Suppose for the moment that illumination is the major factor concerned in diurnal migration. Suppose also that, in agreement with the Weber-Fechner law, the response is a function of the rate of change of the stimulus. Then if we plot curves for equal values of the rate of change of illumination we should have an index of the predicted response in the animal. Consider two animals, one with a day level at 50 fathoms and one at 300. The response values will be high for the deep-living form for a much greater fraction of the day than for the shallow one. In fact, if there is a threshold value of, say 20 (arbitrary units), below which no response is to be expected, the shallow animal will show response for only a short period around sunrise and sunset, while the deep one will have only a brief mid-day period of no response. The same is true to a lesser extent at night. It is obvious that the migrations of a euphausid cannot be explained as simply as this diagram might suggest, since both non-linear response to stimulus and modifying effect of other varying factors must enter into the complex.

Analyses of the bathygrams from the point of view of temperature and illumination have not yet progressed far enough for generalization, but two records are shown in Figure 23a and b as an example of the type of result which is found. The movement of the layer is followed throughout the day, and the temperature is plotted against the illumination at the time and level in question. In Figure 24a there is a period from 04.30 to 05.40 hours when the illumination is increasing, but the layer shows no change of level except for a slight rise at the end of the time. Such a rise just before sunrise is indicated in Russell's diagram (Fig. 10). At an illumination of 10^{-8} the layer abruptly commences to descend, and does so at an accelerating pace until 07.00. During this period it is descending so fast that, despite the fact that the sun's altitude is increasing, the layer is able to attain levels of lower illumination. At an illumination level of about 10^{-12}, descent slows down so as to follow a level of constant illumination for a time, with a gradual transition to a short noon flattening period in which illumination rises, but descent ceases and the temperature remains constant. In the afternoon a similar sequence is followed, but with some hysteresis so that a path of lower illumination is followed throughout.

Figure 23b shows an example in which the noon flattening is more pronounced. The upper levels are missing from this record, but the rest of the pattern is the same except for the sharper break at 08.55. Here the period of decreasing temperature but constant illumination changes over abruptly to one of constant temperature but increasing illumination. There is again a lag in the evening ascent, and here also the change at the commencement of ascent is more abrupt. The relation of these observations to what is known of zooplankton tropisms will not be discussed until more analyses have been made, but two points in particular may be referred to. The hysteresis between the curves for ascent and descent means that the trace of the scattering layer movements will not be quite symmetrical about a midday axis. Also, the fact that the organisms are able to ascend and descend faster than to the iso-illumination curves means that the speed necessary to keep pace with changing illumination is well within the capacity of the animals concerned. Maximum recorded speeds are of the order of one to two fathoms per minute which does not seem excessive for as active an animal as a euphausid. Records from Bermuda (Moore, 1949a) show that the much smaller copepod *Pleuromamma abdominalis* can ascend at a rate of about one fathom per minute.

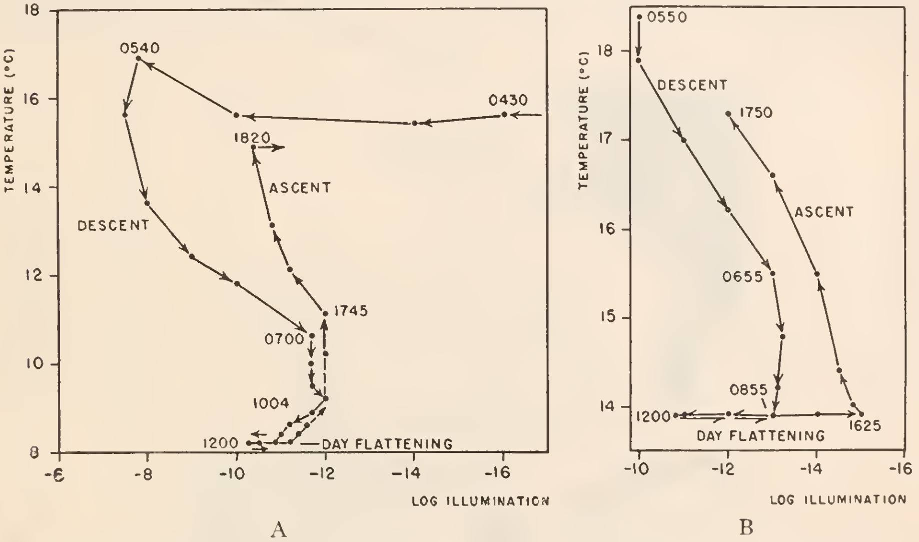


FIGURE 23. Conditions of temperature and illumination experienced by a scattering layer in the course of its diurnal migration. (Figures show local apparent time.) a. *Atlantis* cruise 144, 24 Feb., 1947; 25°26′N., 93°44′W. b. *Atlantis* cruise 144, 13 Jan., 1947; 21°21′N., 75°39′W.

It should be kept in mind that in referring to the effect of a factor on rate of ascent or descent, we do not necessarily infer that the rate of swimming is modified. The mechanism might equally well be one in which an organism, while maintaining a uniform rate of swimming, varied the degree of directness of its somewhat crooked course under the influence of the factor. This may be an important distinction if an attempt is made to explain observed behaviour in terms of experiments on tropisms.

When the euphausids ascend towards the surface at night, it is reasonable to suppose the warmer water encountered may constitute a barrier, just as the cold water did for the day descent. We do not yet have sufficient data on night levels to confirm this, but the sharp night levelling-off in some of the few available records suggests this (cf. Fig. 12). Unlike the day condition, illumination and temperature will, at night, offer barriers in the same direction (that is, to further ascent) and so will not conflict. Since both the phase of the moon, and hence the illumination from it, and also the duration of lunar illumination fluctuate so rapidly, it is probable that any long-term effect such as geographical distribution will reflect surface temperatures more closely than it will night illumination. This is not, of course, true of short-term effects such as the record of migration during a single night. Maximum summer surface temperatures, in so far as they reflect the actual temperatures of the water at the night level of the scattering layer, may therefore be considered as a likely factor limiting the geographical distribution of the species.

Limiting factors in geographical distribution. Since the phenomenon of the scattering layer is of such widespread occurrence, and since its understanding is of such importance in underwater acoustics, the value of any theoretical explanation will be greatly enhanced if it allows prediction of the occurrence and nature of the layer in a given locality. It was found in the Mediterranean that there was some correlation between the intensity of the scattering layer and the density of the euphausid population. It was also found, in a limited area of the Western Atlantic, that there was agreement between the depth of the layer and the dominance of deep- or shallow-living species of euphausids in the population. More extensive data are, of course, needed before either of these points can be considered proven. In this section, an attempt is made to show that the regions of dominance of the various species of euphausid may be defined in terms of the environmental factors of temperature and illumination.

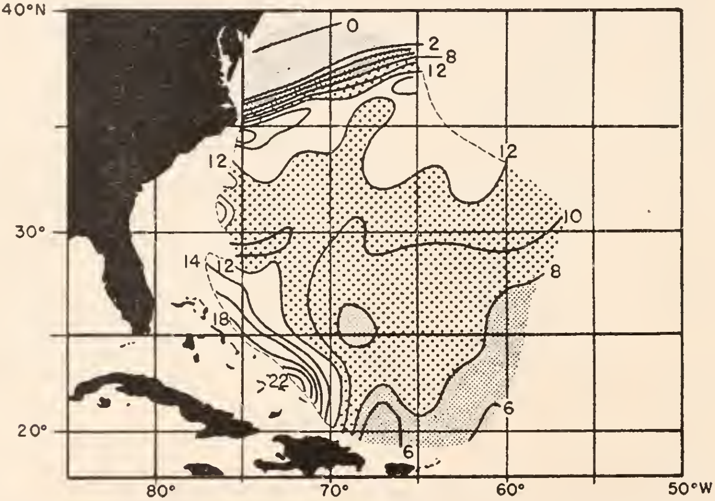


Figure 24. Illumination in summer at depth of 16° isotherm.

The limiting factors concerned in the mid-day levels of the euphausids appear to be a combination of illumination and temperature. If, on account of either low extinction coefficients or low latitudes, illumination is relatively high, then an animal

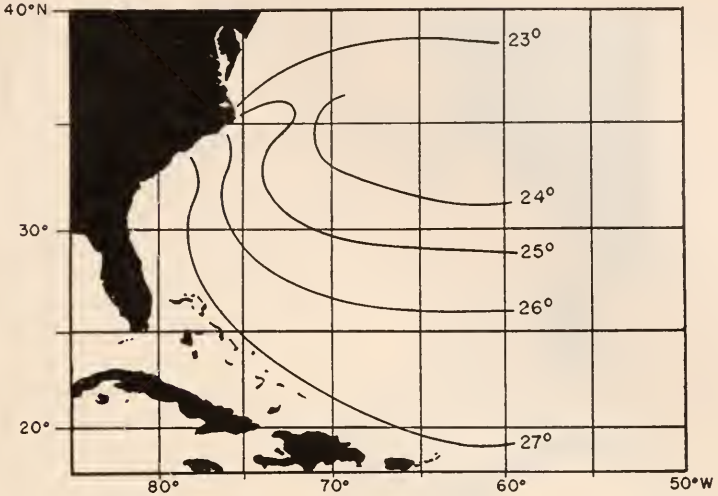

40°N
30°
20°
80°
70°
60°
50°W
23°
24°
25°
26°
27°

Figure 25. Surface temperatures in June. (Mod. from Fuglister, 1947.)

will tend to be forced down into deeper and colder water. Alternatively, if the cold water is near the surface, it may be kept up at a level where the illumination is too high. It may thus be impossible for the animal to find a level where both temperature and illumination are within the tolerance range of the species, and the area must therefore be considered unsuitable for it. In an attempt to express the combined effect of these two factors, we have made use of the illumination at midday, in summer, at the depth of the 16° C. isotherm. This isotherm was chosen

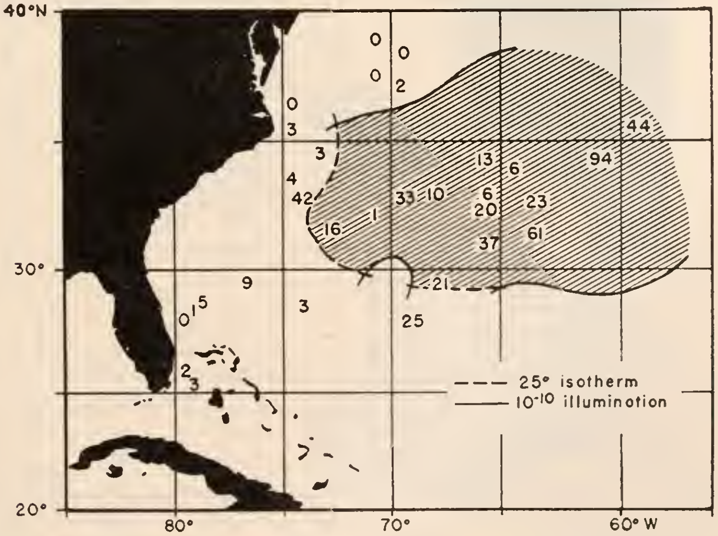

40°N
30°
20°
80°
70°
60° W
0
0
0
2
0
3
3
4
42
44
13
6
94
33
10
6
20
23
1
16
37
61
21
25
9
3
0
1
5
2
3
--- 25° isotherm
—— 10^-10 illumination

Figure 26. Observed distribution of *E. brevis*, and proposed temperature and illumination boundaries.

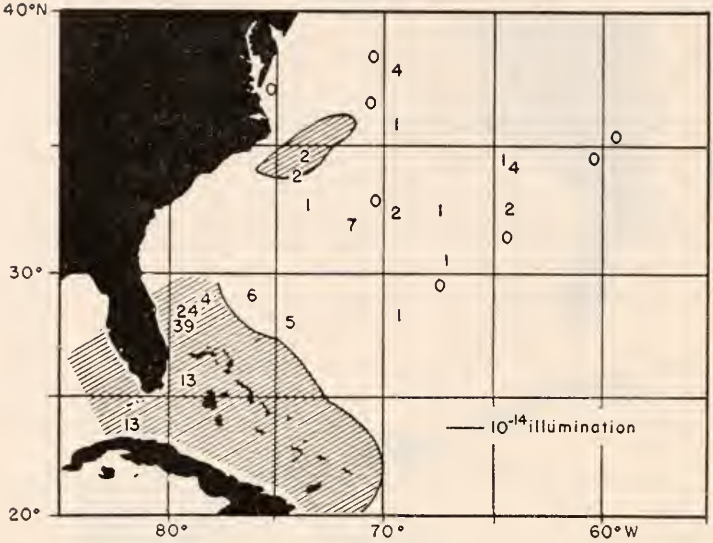

40°N
30°
20°
80°
70°
60°W
10^-14 illumination
0
4
0
1
0
2
2
1
0
2
7
1
1
4
0
0
2
0
1
0
4
24
39
6
5
1
13
13

FIGURE 27. Observed distribution of *E. americana* and proposed boundary of illumination only.

arbitrarily, but, throughout the area of the Northwestern Atlantic which we are considering, it lies at about the depth of the scattering layer. Information on the transparency of the water in the area is scanty, so the chart of illumination at the 16° C. isotherm, shown in Figure 24, is subject to the reservation that it may have to be modified when better data become available.

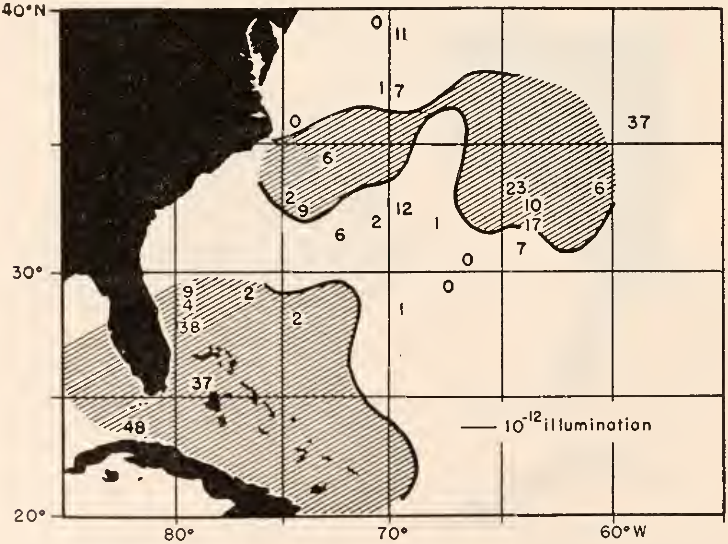

40°N
30°
20°
80°
70°
60°W
10^-12 illumination
0
11
1
7
0
37
6
2
9
6
2
12
1
23
10
17
6
7
0
0
9
4
38
2
2
1
37
48

FIGURE 28. Observed distribution of *E. tenera* and proposed boundary of illumination only.

We have also suggested that at night, when the euphausids are near the surface, they may encounter too high a temperature and be prevented thereby from ascending into the phytoplankton-rich upper waters to feed. The highest temperature encountered will be in summer, and we have used the summer surface isotherms shown in Figure 25 as an index of these surface conditions.

If our choice of limiting factors is correct, it should be possible to select contours from these two charts which will define the areas inhabited by the various species of euphausids. It must be remembered that, in both cases, the curves represent one-way barriers. Surface isotherms represent a limit to spreading towards warmer, but not towards cooler water, while iso-illumination curves represent a barrier to spread into brighter conditions only (lower indices). Figures 26 to 28 show the centers of distribution of three of the commoner species of euphausid in the area, as far as we know them at present, and the values of illumination and temperature which appear to delimit them. The agreement is reasonably good. Data are now being collected from the whole North Atlantic, and the results so far obtained seem to bear out the validity of our choice of limiting factors. If, in this wider area, equally good agreement is obtained between the depth of the scattering layer and that characteristic of the dominant species of euphausid, it is likely that prediction of the depth of the layer for any given locality may be possible.

SUMMARY

The source of the scattering effect is not, at present, susceptible of direct proof, but if an organism could be found whose characteristics agreed with those of the layer on a sufficient number of points, then there would be a high probability that this is the actual source of the effect. From the diurnal migration of the layer there seems no room for doubt that it is caused by an animal. Of the animal population of the sea, three groups seem considerably more probable than any others, namely, fish, squid, and crustacea. There are certain arguments which make the latter the most likely, and if experimental tests now in progress show that the crustacea meet the acoustical requirements, then there will be little reason to consider either fish or squid.

Of the crustacea, the small copepods may prove to be the source of shallow reverberation and possibly some very shallow layers. Euphausid shrimps are the dominant crustacean at the depth of the main scattering layer. These and large red prawns are the dominant crustacea at the depth of a still deeper layer which is briefly referred to. Euphausids occupy the same day level as the main layer, and execute a similar diurnal migration. The dominant species of euphausid varies geographically, and each species has a characteristic day level. Where it has been possible to make a comparison, there is reasonably good agreement between the geographical trend of the daytime depth of the layer and dominance of deep- or shallow-living euphausids. The euphausid population is generally a complex of a number of species. The recording instrument used does not give sufficient definition to show a number of separate layers under such conditions. Certain areas were examined where there was a simpler euphausid population with only two abundant species, and these had different characteristic levels. The bathygrams from this area showed two distinct layers on a number of occasions. The scattering layer is usually confined to deep water, and disappears or is replaced by one with

different characteristics in shallow water. This parallels the distribution of oceanic euphausids, but would probably apply equally well to other oceanic animals. The same is true of the correlation found in the Mediterranean between the decrease eastwards of the scattering effect and the similar decrease of the total plankton, including euphausids. The relation found between the level of the scattering layer and illumination, and the modification of this by temperature, is that which would be predicted for euphausids, but also for other planktonic animals. In one type of scattering layer, a reversed type of diurnal migration has been recorded, the layer descending in the evening and ascending in the morning. It is believed that certain euphausids behave in this way. Such reversal is rare in zooplankton and we do not, at present, know of any other organism which makes as extensive a reversed migration as this particular layer. The characteristics required in an organism which could be the source of the scattering effect are met, in all cases in which they have been tested, by euphausids. They are met in only some cases by other planktonic forms. There seems, therefore, to be a considerable probability that it is euphausids which cause the effect.

In the section on the biology of euphausids, a summary of relevant information for the North Atlantic area is made. On the basis of analyses of the bathygrams, a theory is proposed to explain the limiting action of temperature and illumination as environmental factors. It was found that, subject to the limitations of data at present available, the geographical distributions of various euphausid species, as predicted from hydrographic observations, agreed fairly well with that observed in net hauls. Most previous work on zooplankton has been confined to the shallower-living forms, so attention is drawn to the considerable differences in environmental conditions to which such deeper-living forms as euphausids are subject.

Actual measurements of illumination at these depths have yet to be made. The attempt to compute the illumination and its changes under different conditions is discussed in the Appendix.

At one time and another very many of the staff of the Woods Hole Oceanographic Institution have assisted in obtaining fathograms and other sonic data, hydrographic data, and plankton hauls. Mr. Iselin, Dr. Redfield, Dr. Hersey, Dr. Clarke and others have constantly helped with advice and suggestions. Mrs. Bunker has made many of the calculations and euphausid counts, as well as assisting in the preparation of the paper. To all of these I wish to express my great indebtedness.

Appendix

Illumination computations. Photocell measurements have not been made at as great a depth as that of the scattering layer, so it has been necessary to calculate the illumination there. In doing this certain assumptions have been made for lack of data, and others for simplicity of calculation. An arbitrary value of 100 for overhead sunlight immediately above the water surface, has been taken. This is equivalent to about 10,000 foot candles. In calculating the diurnal changes at a given date and locality, allowance has been made for the effect of varying zenith distance of the sun on the illumination of a given horizontal area. Allowance has also been made for the varying loss by reflection at the surface, using the figures given by Sverdrup (1942, p. 53). No allowance has been made for the varying atmospheric absorption with zenith distance, for degree of cloudiness of the sky, or for sea state. The first of these was disregarded since it varies geographically and seasonally (other than by reason of latitude alone) as well as with atmospheric conditions on a given day. Data given by Haurwitz (1934) indicates that under average conditions, the error caused by this effect will not exceed 20 per cent at zenith distances of less than 60–70°, and 50 per cent at about 80°. Haurwitz (1946) gives figures showing that, with overhead sunlight and ten tenths cloud of maximum density (4), insolation is reduced to 18 per cent. At an air mass of 5.0 (= zenith distance of 78.5°) the figure is 23 per cent. Poole (1936) states that surface loss is unlikely to be increased by waves and introduced bubbles by more than 10–15 per cent. Since our iso-illumination curves are drawn one hundred fold apart, a reduction of surface light of as much as 90 per cent would only mean an error of a half interval in their depth, so it is felt that the above three sources of error may safely be neglected.

Allowing for the angle of refraction of the light entering the water, we have assumed that this angle will be maintained down to the depth in question, and calculated the illumination from the known coefficient of extinction. This will not hold true if a large proportion of the surface illumination comes diffusely from the whole sky rather than directly from the sun, if directional effect is largely lost at the surface because of wave action, or if there is considerable scattering in the water. Kimball (1924) gives mean figures for the ratio of direct solar radiation to that from the sky. For Washington, D. C., these vary from 5.3 : 1 at zenith distances of 25° or less, to 4.1 : 1 at 60°, and 1.6 : 1 at 78.7°. The major fraction of the incident illumination is, then, directional, except at very low sun altitudes; even of the fraction which comes from the sky, that from near the sun is by far the brightest, so there is still a considerable directional quality in it. Whitney (1941) has calculated the mean path length of a ray for different zenith distances and different percentages of sky radiation. At a zenith distance of 60° and average cloud, sky radiation would be 19.5 per cent of the total. From Whitney's values, this would mean that our calculated ray path would be 1.9 per cent too long, which is a negligible error for the present purposes. The error would not exceed 10 per cent at a zenith distance of less than about 80°. Since surface loss by reflection is only 6 per cent at a zenith distance of 60°, and 25 per cent at 80°, the variation in this with cloud or sea state may be neglected. Waves will, in general, tend to decrease the obliquity of the ray and hence its ray path. No data are available on the magnitude of this effect. An estimate of the effect was obtained as follows. The Stokes and Mitchell wave given by Sverdrup *et al.* (1942, p. 527) may be taken as an approximation of the steepest average condition which will be met with. A series of ten parallel rays was considered, spaced equally along a horizontal surface from crest to crest of this wave. The path of each after refraction was calculated, and the mean ray path obtained. Comparison of this, for different solar zenith distances, with ray path for a plane sea surface gave the following results. At Z.D. = 0°, the plane surface path is 1.8 per cent too short, and at 25°, 50°, and 75° it is, respectively, 4.3 per cent, 11.2 per cent and 13.2 per cent too long. Since these figures are based on extreme conditions, it seems justifiable to disregard the effect of sea state on ray path.

Except within about an hour of sunrise and sunset, all the above sources of error may be disregarded. This paper is mainly concerned with illumination at, or near, noon. For detailed study of the periods when the scattering layer is ascending or descending, allowance will have to be made for certain errors. The most serious source of possible error lies in our assumption that a ray, entering the water at a given angle, will maintain this angle to several hundred fathoms. Various workers (*e.g.*, Whitney, 1941), have pointed out that, if there is any scattering in the water, since vertical rays will have a shorter path downwards than oblique ones, there will, therefore, be a progressive removal of all but the vertical ones. Thus, whatever the incident angle at the surface, below a certain depth all light will tend towards vertical, and an angular distribution plot of illumination will be symmetrical about a vertical axis, and with considerably lower intensities away from the vertical. Such an angular shift with depth has been demonstrated in fresh water lakes, and in some marine localities, but never in water with as low an extinction coefficient as that with which we are concerned. For example, in the most transparent water studied by Whitney (1941) there was an angular shift from about 30° at 10 metres to 25° at 40 metres. From the location, 39°34′N., 71°05′W., one would assume that the extinction coefficient was in the neighborhood of 0.10. Very little of our area has a coefficient approaching this, and in some parts it falls below 0.04. Jenkins (1946) gives data on the relative proportions of scattering and absorption in the attenuation of light in sea water. While the two may be of the same order of magnitude in coastal waters, he considers that scattering is negligible in oceanic waters, except, perhaps, in the top hundred metres. Since we are concerned almost exclusively with oceanic water, it seems reasonable to assume that, if any reduction in obliquity of the light takes place, the reduction will be mainly near the surface, and will be of a lower order than that shown by Whitney (1941, Fig. 5). Until actual measurements are available of the illumination at a depth of several hundred fathoms, we feel that the assumption that there is relatively slight change in obliquity of the rays in the open ocean gives the best approximation to actual conditions. Any error so introduced will be greatest towards sunrise and sunset, and will result in our iso-illumination curves being drawn too close to the surface.

Extinction coefficient data have been collected from all available sources, and charted. There are still so few of these that we feel that inaccuracies in the coefficients which we have made use of are likely to be the most serious source of error in the computation of the illumination curves, particularly as a small difference in extinction coefficient in this very clear water gives rise to a considerable change in illumination at three hundred fathoms. It is unlikely that we are in error by as much as 0.01, except in areas such as the edge of the Gulf Stream, and at the noon depth of the scattering layer this would give rise to an error in the calculated illumination of about 10^{-2}.

The possibility that extinction coefficients decrease considerably with increasing depth in the open ocean is one which requires consideration. It may well necessitate adjustment of our deeper figures. Certain theoretical considerations suggest its probability, but so far any direct measurements are lacking.

LITERATURE CITED

Bogorov, B. G., 1946. Peculiarities of diurnal vertical migrations of zooplankton in Polar seas. *J. Mar. Res.*, **6**: 25–32.

Bumpus, D. F., 1948. Notes on the productivity of the Mediterranean Sea. (Unpublished.) Presented 11 September, 1948 at Am. Soc. Limn. and Oceanogr. meeting.

Cattell, J. McK., 1935. The biological importance of pressure. *Sci. Monthly,* **40**: 468–475.

Cattell, J. McK., 1936. The physiological effects of pressure. *Biol. Rev.,* **11**: 441–476.

Clarke, G. L., 1933. Diurnal migration of plankton in the Gulf of Maine and its correlation with changes of submarine irradiation. *Biol. Bull.,* **65**: 402–436.

Dietz, R. S., 1948. Deep scattering layer in the Pacific and Antarctic Oceans. *J. Mar. Res.,* **7**: 430–442.

Einarsson, Hermann, 1945. *Euphausiacea.* I. Northern Atlantic species. *Dana Rept.,* **27**: 1–185.

Fuglister, F. C., 1947. Average monthly sea surface temperatures of the western North Atlantic Ocean. *Pap. Phys. Oceanogr. and Meteorol.,* **10**: 1–25.

Hardy, A. C. and W. Neil Paton, 1947. Experiments on the vertical migration of plankton animals. *J. Mar. Biol. Assoc.,* **26**: 467–526.

Haurwitz, Bernhard, 1934. Daytime radiation at Blue Hill Observatory in 1933 with application to turbidity in American air vessels. *Harvard Met. Stud. No. 1.*

Haurwitz, Bernhard, 1946. Insolation in relation to cloudiness and cloud density. Blue Hill Observatory, Harvard Univ., Milton, Mass. Repr. No. **4**: 154–166.

Helge, Thomsen, 1931. Nitrate and phosphate contents of Mediterranean water. *Rept. Dan. Oceanogr. Exped. 1908–1910,* **3**: 1–14.

Hersey, J. B. and H. B. Moore, 1948. Progress report on scattering layer observations in the Atlantic Ocean. *Trans. Am. Geophys. Un.,* **29**: 341–354.

Jenkins, F. A. and I. S. Bowen, 1946. Transparency of ocean water. *J. Opt. Soc. Am.,* **36**: 617–623.

Jespersen, P., 1935. Quantitative investigations on the distribution of macroplankton in different oceanic regions. *Dana Rept.,* **7**: 1–44.

Johnson, M. W., 1948. Sound as a tool in marine ecology, from data on biological noises and the deep scattering layer. *J. Mar. Res.,* **7**: 443–458.

Kikuchi, K., 1930. Diurnal migration of plankton crustacea. *Quart. Rev. Biol.,* **5**: 189–206.

Kimball, H. H., 1924. Records of total solar radiation intensity and their relation to daylight. *Monthly Weather Rev.,* **52**: 473–479.

Leavitt, B. B., 1935. A quantitative study of the vertical distribution of the larger zooplankton in deep water. *Biol. Bull.,* **68**: 115–130.

Leavitt, B. B., 1938. The quantitative vertical distribution of macrozooplankton in the Atlantic Ocean basin. *Biol. Bull.,* **74**: 376–394.

Lyman, G., 1948. The sea's phantom bottom. *Sci. Monthly,* **66**: 87–88.

Moore, H. B., 1948. Report on the biological interpretation of the deep scattering layer in the North Atlantic. *Rept. to U. S. BuShips,* pp. 1–30.

Moore, H. B., 1949a. The zooplankton of the upper waters of the Bermuda area of the North Atlantic. *Bull. Bingham Oceanogr. Coll.,* **12**: 1–97.

Moore, H. B., 1949b. *Atlantis* cruise 151 to the Mediterranean area. *Sci. Rept. No. 3,* Scattering layer observations, pp. 1–8. Woods Hole Oceanographic Institution Ref. No. 49–2.

Moore, H. B., 1949c. A camera for producing one-dimensional reduction in narrow records. *J. Mar. Res.,* **7**: 21–23.

Poole, H. H., 1936. The measurement of submarine light and its relationship to biological phenomena. *Rapp. et Proces-Verb.,* **101**: 1–8.

Raitt, R. W., 1948. Sound scatterers in the sea. *J. Mar. Res.,* **7**: 393–409.

Reynard, P., 1885. Phénomènes objectifs que l'on peut observer sur les animaux soumis aux hautes pressions. *C. R. Soc. Biol. Paris,* **37**: 510–515.

Russell, F. S., 1927. The vertical distribution of plankton in the sea. *Biol. Rev.,* **2**: 213–262.

Russell, F. S., 1931. The vertical distribution of marine macroplankton. XI. Further observations on diurnal changes. *J. Mar. Biol. Assoc.,* **17**: 767–784.

Ruud, J. T., 1936. Euphausiacea. *Rept. Dan. Oceanogr. Exped. 1908–1910,* **2**: 1–86.

Sverdrup, H. U., 1942. Oceanography for Meteorologists. Prentice-Hall, Inc., New York. 235 pp.

Tattersall, W. M., 1926. Crustacea of the orders Euphausiacea and Mysidacea from the Western Atlantic. *Proc. U. S. Nat. Mus.,* **69**: 1–28.

Townsend, C. H., 1935. The distribution of certain whales as shown by logbook records of American whaleships. *Zoologica* (N. Y.), **19**: 1–50.

UNIVERSITY OF CALIFORNIA, 1942. Reverberation studies at 24 kc. *Division of War Research Rept.* No. U–7.

UNIVERSITY OF CALIFORNIA, 1943. Volume reverberation; scattering and attenuation versus frequency. *Division of War Research Rept.* No. U–50.

UNIVERSITY OF CALIFORNIA, 1946. Forward scattering from the deep scattering layer. *Division of War Research Rept.* No. M–398.

UNIVERSITY OF CALIFORNIA, 1946. Studies of the deep scattering layer. *Division of War Research Rept.* No. M–445.

WATERMAN, T. H., R. F. NUNNEMACHER, F. A. CHASE AND G. L. CLARKE, 1939. Diurnal migration of deep water plankton. *Biol. Bull.,* **76**: 256–279.

WHITNEY, L. V., 1941. A general law of diminution of light intensity in natural waters and the percent of diffuse light at different depths. *J. Opt. Soc. Am.,* **31**: 714–722.

THE RESPIRATORY METABOLISM OF EXCISED TISSUES OF WARM- AND COLD-ADAPTED FISHES

C. N. PEISS[1] AND JOHN FIELD[2]

Arctic Research Laboratory, Office of Naval Research, Point Barrow, Alaska, and Department of Physiology, Stanford University School of Medicine, Stanford, California

A striking characteristic of certain arctic poikilotherms such as the arctic (or polar) cod, blackfish, sculpin and indigenous isopods and amphipods is their ability to remain active at environmental temperatures ranging around 0° C. or lower. In the case of the fish there is good reason to believe that body temperature is within a few tenths of a degree of the temperature of the water habitat (Clausen, 1934; Gunn, 1942). Polar cod are often found in sea water in ice pockets and cracks where the water temperature is below 0° C. In contrast, many poikilothermic forms living in the temperate zone are in a state of cold narcosis at such temperature (Parker, 1939). In consonance with these observations is the finding that failure of conduction occurs in the sciatic nerve of the green frog at about 5° C. (Gasser, 1931).

Recently Irving and his associates (Edwards and Irving, 1943a, 1943b; Haugaard and Irving, 1943) described a form of adaptation to temperature in certain non-hibernating poikilotherms. They showed that in these animals the effect of temperature on metabolic rate depends in part on the temperature of the environment from which the animal was taken. Thus, at temperatures below 20° C., the oxygen consumption of the sand crab (*Emerita talpoida,* Say), after allowance for differences in size, was greater in winter than in summer. Both the temperature of maximum oxygen uptake and the thermal death point (range) were lower in winter than in summer (Edwards and Irving, 1943a). This change in the properties of the biological oxidation system on long exposure to cold may be regarded as a primitive form of chemical defense, and the continued growth and activity of the sand crab during the winter indicate that it is an important adjustment of the animal to season. No such seasonal influence on the effect of temperature on oxygen consumption was found in the beach flea (*Talorchestia megalopthalma*), an air-breathing neighbor of the sand crab (Edwards and Irving, 1943b). In line with these differences, the beach flea, instead of remaining active during the winter, went into a state of apparent hibernation beneath the sand.

In the cunner (*Tautologolabrus adspersus,* Walbaum) there was some adaptation of the oxidative metabolism to season, but this was insufficient to allow the necessary physical activity to enable the cunner to remain in the summer habitat during the winter months. This view agrees with the disappearance of the cunner from the shoreline waters in winter (Haugaard and Irving, 1943).

---

[1] Present address: Dept. of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

[2] Present address: Ecology Branch, Office of Naval Research, Navy Department, Washington, D. C.

It has long been established that poikilotherms can be acclimatized to withstand environmental temperatures that are normally lethal (Davenport and Castle, 1896; Vernon, 1899–1900; Loeb and Wasteneys, 1912). The relationship between environmental temperature and lethal temperature has been defined more precisely in a number of acclimatization studies by Hathaway (1927), Fry, Brett and Clawson (1942), Doudoroff (1942, 1945) and Brett (1944). Earlier work has provided other examples of metabolic adaptation to temperature in poikilotherms (Battle, 1926; Britton, 1930; Barcroft, 1934; Fox, 1939) and in hibernating homoiotherms (Tait, 1922; Britton, 1930; Suomalainen, 1939). Wells (1935) demonstrated a variation of respiratory metabolism of certain fish with season, and showed that the oxygen consumption was high in the late winter months and low during the summer months. He concluded that "it seems certain that there is some adaptation to high and low temperatures in fish."

On the basis of the evidence in hand it seems reasonable to assume that the phenomenon of metabolic adaptation to temperature is a rather general one. However, no evidence is presently available as to the nature of this effect. The present work was designed to provide data on the tissue metabolism of cold- and warm-adapted forms in relation to environmental temperature, which might contribute to the elucidation of the marked tolerance for cold shown by arctic cod and provide further information as to the nature of metabolic adaptation to temperature.

METHODS

The work dealing with the cold-adapted fish was performed at the Arctic Research Laboratory, Point Barrow, Alaska (Shelesnyak, 1948). The form chosen was the polar cod, *Boreogadus saida,* Lepechin (Jordan and Evermann, 1898; Jordan, 1905, 1923), which is found in large schools around the edges and in crevasses of floating and pack ice. Since the shoreline at Point Barrow is ice-free for only a short time each year, it is necessary to utilize the available time to the fullest. Accordingly, during the period of six weeks when the ice was relatively close to shore, large numbers of fish were caught and maintained in aquaria in the laboratory.[3] While the polar cod is occasionally available at other times by fishing through the ice, the obvious difficulties of extreme cold and ice thickness make procurement very difficult. The water temperature in the habitat of the fish at the time of capture varied from − 1.5 to + 2.0° C. The fish were maintained in the laboratory in aerated aquaria at temperatures ranging from − 1.0 to 0° C.

The experiments with the Golden Orfe, *Idus melanotus* (Heckel and Kner, 1858; Guenther, 1868, 1880; Buiytendijk, 1910), were all carried out in the laboratories of the Department of Physiology at Stanford University. These fish were obtained from a commercial dealer, who maintained the aquarium temperature at 25° C. for a minimum period of one week. Suitable numbers of these fish were transferred periodically to the laboratory aquarium, where they were maintained at 25° C. until used. In general, the fish had been living at a 25° C. environmental temperature for two weeks before use.

---

[3] We wish to express our appreciation to Professor George MacGinitie, Director, Arctic Research Laboratory, Point Barrow, Alaska, and to his crew of Eskimos for their efforts in obtaining the fish for our use.

The following procedures and methods apply to both species of fish. The fish were decapitated, and the brain and liver were rapidly excised. Since the physical properties of the tissue precluded the preparation of brain slices, the whole brain was finely minced with scissors. Liver slices were prepared by the Lucite template method (Crismon and Field, 1940) by means of a clean dry safety razor blade (Field, 1948). The moist cold box technique was used throughout (Peiss and Field, 1948; Field, 1948). Respiration was measured by the Warburg manometric method (Dixon, 1943; Umbreit, Burris and Stauffer, 1945). The gas phase was oxygen. The center wells of the respirometer flasks contained 10% KOH with Whatman No. 40 filter paper wicks. The liquid phase was Ringer-phosphate, pH 7.4 (glass electrode). Manometric measurements were made in a constant temperature bath, equipped with a refrigerator unit, over a temperature range of 0° to 25° C. At any given temperature, the water bath was maintained at ± 0.02° C. Readings were taken at intervals of 10–20 minutes, depending on the respiratory rate of the tissue at the temperature used, and were carried out for a minimum of 120 minutes. Results are expressed in terms of wet weight Q_{O_2}. Thus Q_{O_2} (wet wt.) denotes microliters of oxygen consumed, measured under standard conditions, per milligram initial wet weight of tissue per hour.

Tissue water content was determined by drying to constant weight at 103° C. The mean water content and range, in per cent, for *B. saida* were: brain, 80.5, range 79.4–82.0, 26 fish; liver, 55.2, range 48.2–70.4, 24 fish. The values for *I. melanotus* were: brain, 78.4, range 76.4–80.4, 18 fish; liver, 74.6, range 71.4–76.8, 14 fish. The large range in *B. saida* liver appears to be due to variable amounts of oil residue in the tissue.

RESULTS

1. *Physical measurements*

The polar cod was chosen for this study for a number of reasons, chief of which were the relatively high activity of the animals at low temperature, the large number of animals available and the relative ease with which they were obtainable. The Golden Orfe was selected as the warm-adapted fish primarily because it was the most readily available fish, in large numbers, which could withstand the high environmental temperature, and which was similar in body size to the polar cod. While the cod is a marine fish and the Orfe a fresh water form, the sea habitat of the cod we used was one of low salinity, and it has been shown that this species of cod survives readily in fresh water. Table I summarizes the body weight and length data for the two species.

TABLE I

Body weight and length data for polar cod (B. saida) and Golden Orfe (I. melanotus)

	B. saida		*I. melanotus*	
	Weight, gms.	Length, cm.	Weight, gms.	Length, cm.
Mean	21.1	15.5	16.8	13.0
Range	9.8–32.0	10.0–20.5	7.3–25.2	8.5–16.5
Std. dev.	4.7	2.2	3.8	1.9
No. fish	90	90	94	94

For both species of fish, the wet weight of the brain was very similar, averaging about 120 milligrams. There was, however, a wide discrepancy in the size of the liver. In the Orfe, this organ was rather diffuse, and was made up, for the most part, of three long, slender lobes. The average weight for the liver of the Orfe was of the order of 200–300 mg. The liver of the polar cod, in contrast, was large in comparison to the total weight of the whole fish. In most cases it weighed from 750–900 mg., but it was not uncommon to find a liver weighing more than a gram in a fish whose total body weight was on the order of 20 grams.

2. *Time course experiments*

An extensive series of experiments were made to determine the effect of time on oxygen consumption over the temperature range 0–25° C. In the case of brain and liver tissue of the Orfe, oxygen consumption was a linear function of time at all temperatures for 180–240 minutes, which was the maximum time of observation. The data for the polar cod were more variable with regard to temperature. These data are shown in Table II.

TABLE II

Duration of steady states of oxygen consumption of polar cod brain and liver at graded temperatures. Periods marked with asterisks are those in which oxygen consumption was still constant when readings were terminated

Temperature, °C.	Brain mince	Liver slices
0	300 min.*	360 min.*
5	300 min.*	360 min.*
10	240–270 min.	360 min.*
15	170–210 min.	200–240 min.
20	90–140 min.	120–150 min.
25	10– 50 min.[a]	20– 60 min.[a]

[a] At this temperature there was wide variation in the constancy of oxygen consumption with time. This was more pronounced with the brain mince. See text for further discussion.

It can readily be seen that as the temperature is increased the period of time during which oxygen consumption is constant decreases, this effect being more pronounced with the brain mince. At 25° C. it was possible to obtain satisfactory readings for at least 40 minutes in most preparations of liver slices. However, when brain mince was used at this temperature, many of the runs resulted in die-away curves from zero time. It was possible to obtain 3 readings at 10 minute intervals, during which oxygen consumption was constant, in only about 50 per cent of the runs. Thus, it was considered advisable to represent the oxygen consumption of polar cod brain mince at 25° C. by two figures, one representing the mean value for all determinations on the basis of a one hour period, and the other representing the mean value calculated from those runs in which oxygen consumption was constant for 30 minutes or more. This will be pointed out again when Table III and Figure 1 are discussed.

3. *Experiments with polar cod and Golden Orfe brain mince*

The oxygen consumption of brain mince from polar cod (*B. saida*) and Golden Orfe (*I. melanotus*) was determined at temperatures ranging from 0°–25° C., at

5° intervals. Approximately 100 fish of each species were used, and from 15–30 determinations were made for a given temperature. These data, together with certain statistical measures, are shown in Table III.

The upper figure at 25° C. for *B. saida* is taken from the portion of the oxygen uptake curves of those determinations that were constant for 30 minutes or more. The lower figure represents the overall mean for all determinations over a period of one hour. All other figures are based on readings taken from steady state oxygen uptake. Figure 1 illustrates the data graphically. The upper solid circle at 25° C., is taken from the upper figure in Table III, and the lower solid circle from the lower figure in Table III.

TABLE III

Respiration of B. saida and I. melanotus brain mince at graded temperatures

B. saida (arctic cod)

Temp., ° C.	0	5	10	15	20	25
Mean Q_{O_2} wet wt.	.267	.356	.523	.794	1.128	1.649* 1.252
Range	.217– .309	.309– .418	.418– .666	.732– .855	.928– 1.382	.817– 1.513
Standard deviation	.027	.029	.064	.035	.116	.188
Number runs	30	26	30	26	26	18
Number fish	14	21	15	17	15	8

I. melanotus (Golden Orfe)

Temp., ° C.	0	2.5	5	7.5	10	15	20	25
Mean Q_{O_2} wet wt.	.078	.114	.137	.203	.361	.602	.926	1.370
Range	.051– .109	.094– .133	.110– .165	.176– .231	.277– .480	.504– .707	.700– 1.181	1.131– 1.702
Standard deviation	.017	.014	.015	.017	.048	.056	.132	.162
Number runs	17	15	18	15	18	18	18	18
Number fish	10	10	15	13	13	12	11	10

* This value obtained if first 30 minutes are used for calculating (see text).

When the logarithm of oxygen consumption, expressed as Q_{O_2}, is plotted against temperature in degrees centigrade, several interesting features are apparent from the curves obtained. Neglecting the 25° C. point for *B. saida,* the cold-adapted fish (solid circles), log oxygen consumption appears to be a rectilinear function of temperature. The dotted line represents the line of "best fit" as calculated by the method of least squares (Snedecor, 1946). The regression equation is:

$$\text{Log } Q_{O_2} = -0.59 + 0.0318t.$$

The Q_{10}, calculated from this equation, is 2.08.

The curve for *I. melanotus* (open circles) appears to be rectilinear over the range 10–25° C., although it is not possible to decide just where the slope starts to change. The regression line for the rectilinear portion of the curve (dotted line) is:

$$\text{Log } Q_{O_2} = -0.81 + 0.0386t.$$

The Q_{10} calculated therefrom is 2.43.

The most striking aspect of the curves in Figure 1 is the sharp break in the lower curve below 10° C. Such a break indicates an increase in the Q_{10} for the values below 10° C., and does not appear in the curve for the polar cod (Table V). The possible significance of this difference will be discussed later.

4. Experiments with polar cod and Golden Orfe liver slices

The overall data, and certain statistical measures, for the oxygen consumption of liver slices of polar cod and Orfe are shown in Table IV. Figure 2 illustrates the data graphically as with the brain mince experiments. The curves resulting from a plot of log oxygen consumption against temperature reveal the same pattern

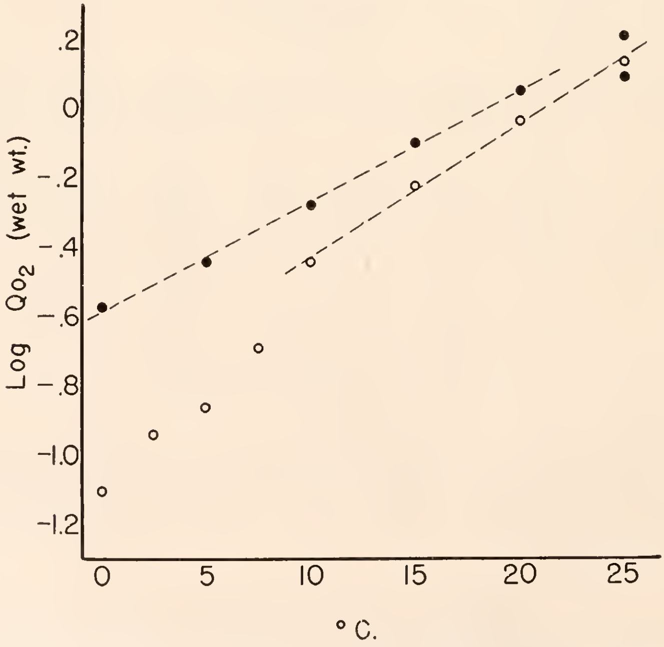


FIGURE 1. Logarithm of oxygen consumption in brain mince of *Boreogadus saida* (solid circles) and *Idus melanotus* (open circles) as a function of temperature.

TABLE IV

Respiration of B. saida and I. melanotus liver slices at graded temperatures

B. saida (arctic cod)

Temp., ° C.	0	5	10	15	20	25
Mean Q_{O_2} wet wt.	.137	.186	.269	.413	.604	.859
Range	.108– .169	.159– .237	.204– .346	.372– .475	.530– .650	.340– 1.093
Standard deviation	.016	.020	.040	.017	.042	.248
Number runs	30	26	34	26	26	18
Number fish	14	21	15	17	15	8

I. Melanotus (Golden Orfe)

Temp., ° C.	0	2.5	5	7.5	10	15	20	25
Mean Q_{O_2} wet wt.	.057	.077	.117	.171	.232	.369	.552	.792
Range	.043– .071	.056– .104	.085– .142	.141– .193	.200– .261	.297– .433	.411– .640	.643– .914
Standard deviation	.010	.017	.016	.017	.017	.040	.066	.080
Number runs	17	15	18	15	18	18	18	18
Number fish	10	10	15	13	13	12	11	10

as that seen above with brain mince (Fig. 1). The regression equation for the cold-adapted polar cod (solid circles) is:

$$\text{Log } Q_{O_2} = -0.88 + 0.0324t.$$

And the Q_{10} calculated from this equation is 2.11. The regression equation for the warm-adapted Golden Orfe (open circles) is:

$$\text{Log } Q_{O_2} = -0.97 + 0.0352t$$

with a Q_{10} equal to 2.25 over the range 10–25° C. Again, the curve for the cold-adapted form (*B. saida*) appears to be rectilinear over the entire temperature range 0–25° C., while that for the warm-adapted form (*I. melanotus*) is rectilinear only over the range 10–25° C. As in the experiments with brain mince from these two species, the Q_{10} increases sharply for the warm-adapted fish below 10° C. (Table V). Thus, although the respiratory rate of the liver slices is of the same order of magnitude at temperatures of 10° C. and higher, the arctic cod liver respires at progressively higher rate, relative to that of Orfe liver, as the temperature decreases from 10° C. to 0° C. In both brain mince and liver slice, the respiratory rate of

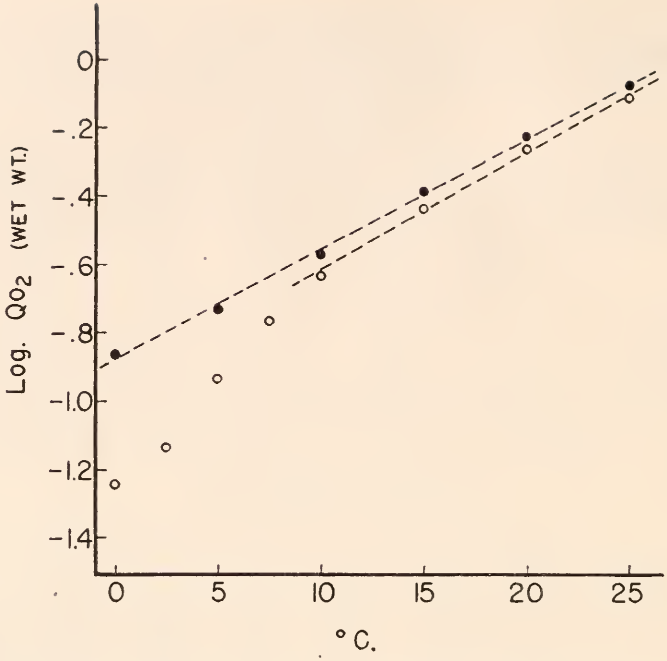


FIGURE 2. Logarithm of oxygen consumption in liver slices of *Boreogadus saida* (solid circles) and *Idus melanotus* (open circles) as a function of temperature.

the tissues from the polar cod is two to three times that of the respective tissues from the Orfe at 0° C. Essentially the two tissues from each species show the same pattern of respiratory metabolism as a function of temperature, but the pattern differs as between species.

TABLE V

Q_{10} values calculated from data in Tables III and IV

Brain mince				Liver slice			
B. saida		*I. melanotus*		*B. saida*		*I. melanotus*	
Temp.	Q_{10}	Temp.	Q_{10}	Temp.	Q_{10}	Temp.	Q_{10}
0–5	1.78	0–2.5	4.57	0–5	1.84	0–2.5	3.33
5–10	2.16	2.5–5	2.09	5–10	2.09	2.5–5	5.32
10–15	2.30	5–7.5	4.82	10–15	2.36	5–7.5	4.57
15–20	2.02	7.5–10	9.99	15–20	2.14	7.5–10	3.39
20–25	—	10–15	2.78	20–25	2.02	10–15	2.53
		15–20	2.37			15–20	2.24
		20–25	2.19			20–25	2.06
Reg. line 0–20	2.08	Reg. line 10–25	2.43	Reg. line 0–25	2.11	Reg. line 10–25	2.25

The Q_{10} values calculated from the data in Tables III and IV are given below in Table V, as well as the Q_{10} values obtained from the regression line over that portion of the curve that is rectilinear.

Discussion

The only other work which we may compare directly with our results is that of Fuhrman and co-workers (1944) on the metabolism of excised brain of the large mouthed bass, *Huro salmoides*. Figure 3 is taken from the results of these investigators.

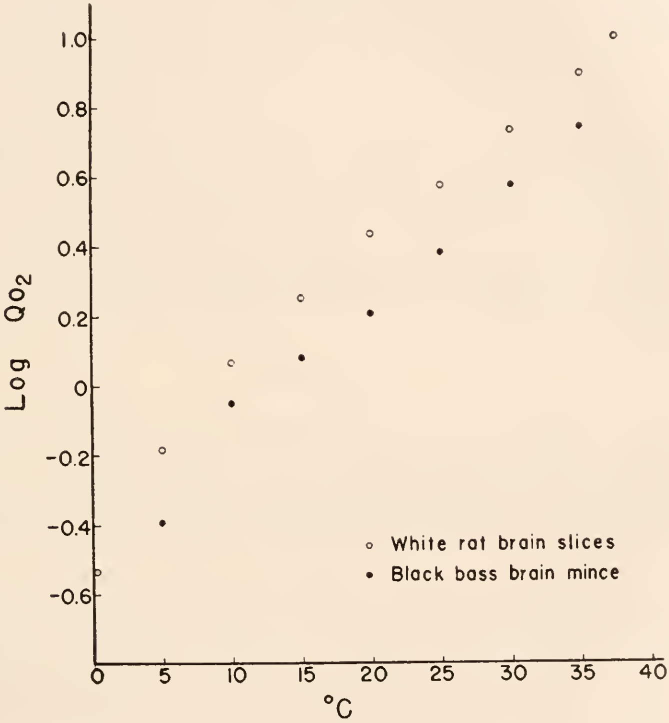


FIGURE 3. Logarithm of oxygen consumption of white rat brain slices (open circles) and black bass brain mince (solid circles) as a function of temperature.

It is apparent from inspection of this figure that the general pattern is similar to that of *I. melanotus* brain and liver. The Q_{10}'s, respectively, for the rat and bass brain over the range 10–35° C., are 2.14 and 2.06. In each case, over the range 0–10° C., there is an abrupt increase in Q_{10} to the order of 4–5. Thus, the only case we have found where Q_{10} remains essentially constant at the lower temperature is with brain and liver tissue from an arctic-adapted form, the polar cod.

Close inspection of Figures 1 and 2, in which log Q_{O_2} is plotted against temperature, reveals that the log Q_{O_2} may not truly be a rectilinear function of temperature. Other workers have pointed out examples where the rule of van't Hoff does not adequately express the data. Wells (1935a) measured the respiratory metabolism of Fundulus at graded temperatures and found that the increased oxygen consumption with a rise in temperature was more pronounced at the lower temperatures. He concluded that the data could not be satisfactorily expressed either by the rule of van't Hoff or by the Arrhenius equation. For a discussion of temperature coefficients, see Belehradek (1930, 1935), Crozier (1924), Sizer (1943), Stearn (1949) and Wilson (1949). Similar results were obtained by Ege and Krogh (1915–16) in their studies on oxygen consumption of goldfish. These workers reported a Q_{10} of 9.8 for the range 0–5° C., 3.8 for the range 5–10° C., and over the range 10–28° C. the Q_{10} decreased from 2.9 to 2.2. Both cases, however, appear to follow the general pattern we have seen in tissues of *I. melanotus,* bass and albino rat, as do the results of Gasser (1931, 1933), working on temperature coefficients of spike potential, refractory period and conduction velocity in amphibian nerve.

The present observations show that the arctic cod has at least two advantages over the warm-adapted fish in adaptation to cold. First, at low temperatures the oxygen consumption per unit weight of brain and liver is several times as great; second, the temperature coefficient for oxygen consumption in brain and liver does not rise below 10° C. (as in the Orfe adapted to 25° C. and the bass adapted to 18° C.). Both the high Q_{O_2} and constant rather than increasing temperature coefficient at low temperatures are metabolic features of value in arctic adaptation.

This work was performed under a contract with the Ecology Branch, Office of Naval Research.

SUMMARY

1. Respiratory metabolism has been studied in brain and liver tissues of two species of fish of similar mean body size, the arctic-adapted polar cod (*Boreogadus saida*), living at environmental temperatures of − 1.5 to + 2.0° C., and the Golden Orfe (*Idus melanotus*), living at an environmental temperature of 25° C. Experiments were carried out in the Warburg apparatus over the range 0–25° C.

2. Oxygen consumption in tissues of the Orfe was constant at all temperature levels studied for 180–240 minutes. In tissues of the polar cod, oxygen consumption was constant for 5–6 hours in the temperature range 0–5° C., and then the duration of the steady state decreased progressively as the temperature increased, so that at 25° C. constant oxygen consumption was observed in most cases for no longer than 40 minutes.

3. The Q_{10} for the steady state respiration of tissues of these two species of fish is of the same order of magnitude; values obtained were 2.08 and 2.11, respectively, for polar cod brain and liver, and 2.43 and 2.25 for Golden Orfe brain and liver. However, the Q_{10} remains essentially constant in the case of tissues of polar cod over the entire temperature range studied, whereas it increases sharply in Orfe tissues in the range 0–10° C. Thus, at low temperatures, the oxygen consumption of tissues of the cod, per unit weight, is several times as great as in the Orfe. These relationships are seen more strikingly when the logarithm of oxygen consumption is plotted against reciprocal of absolute temperature, or when the data are analyzed according to the Arrhenius equation.

4. These findings are discussed in relation to the problem of temperature adaptation in poikilotherms. It is concluded that the differences in response of tissues from the polar cod, compared with those of warm-adapted fish, are advantageous in adaptation of the organism to cold.

LITERATURE CITED

Barcroft, J., 1934. Features in the architecture of physiological function. Cambridge University Press, Cambridge.

Battle, H. I., 1926. Effects of extreme temperatures on muscle and nerve tissue in marine fishes. *Trans. Roy. Soc. Canada,* **20**: Sect. V: 127–143.

Belehradek, J., 1930. Temperature coefficients in biology. *Biol. Revs.,* **5**: 30–58.

Belehradek, J., 1935. Temperature and living matter. Protoplasma Monographien, Vol. **8**, Borntraeger, Berlin.

Brett, J. R., 1944. Some lethal temperature relations of Algonquin Park fishes. Publication of the Ontario Fisheries Research Laboratory, No. 63. *University of Toronto Studies. Biological Series* No. **52**: 5–49.

Britton, S. W., 1930. Seasonal variations in survival after adrenalectomy. *Am. Jour. Physiol.,* **94**: 686–691.

Buiytendijk, F. J. J., 1910. Over het zuurstoff-verbruik van het zenuwstelsel. *Koninklin. Akad. Wetensch. Amsterd. Vergad. Wis. Natuurk. Afd.,* **19**: 615–621.

Clausen, R. G., 1934. Body temperature of fresh water fishes. *Ecology,* **15**: 139–144.

Crismon, J. M. and J. Field, 1940. The oxygen consumption *in vitro* of brain cortex, kidney and skeletal muscle from adrenalectomized rats. *Am. Jour. Physiol.,* **130**: 231–238.

Crozier, W. J., 1924. On biological oxidations as function of temperature. *Jour. Gen. Physiol.,* **7**: 189–216.

Davenport, C. B. and W. E. Castle, 1896. Studies in morphogenesis. III. On the acclimatization of organisms to high temperatures. *Arch. Entwickelungsmechanik der Organismen,* **2**: 227–249.

Dixon, M., 1943. Manometric methods. Macmillan Co., New York.

Doudoroff, P., 1942. The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with *Girella nigricans* (Ayres). *Biol. Bull.,* **83**: 219–244.

Doudoroff, P., 1945. The resistance and acclimatization of marine fishes to temperature changes. II. Experiments with Fundulus and Atherinops. *Biol. Bull.,* **88**: 194–206.

Edwards, G. A. and L. Irving, 1943a. The influence of temperature and season upon the oxygen consumption of the sand crab, *Emerita talpoida* Say. *Jour. Cell. Comp. Physiol.,* **21**: 169–182.

Edwards, G. A. and L. Irving, 1943b. The influence of season and temperature upon the oxygen consumption of the beach flea, *Talorchestia megalopthalma. Jour. Cell. Comp. Physiol.,* **21**: 183–189.

Ege, R. and A. Krogh, 1915–16. On the relation between the temperature and the respiratory exchange in fishes. *Internat. Rev. d. ges. Hydrobiol. u. Hydrog.,* **7**: 48–55.

Eyring, H., 1935. The activated complex in chemical reactions. *Jour. Chem. Physics,* **3**: 107–115.

Field, J., 1948. Respiration of tissue slices. *In* Methods in Medical Research, Vol. 1, Yearbook Pub., Chicago, pp. 301–303.

Fox, H. M., 1939. The activity and metabolism of poikilothermal animals in different latitudes. *Proc. Zool. Soc. London,* Series A, **109**: 141–156.

Fry, F. E. J., J. R. Brett and G. H. Clawson, 1942. Lethal limits of temperature for young goldfish. *Rev. Canadienne de Biol.,* **1**: 50–56.

Fuhrman, F. A., N. Hollinger, J. M. Crismon, J. Field and F. W. Weymouth, 1944. The metabolism of the excised brain of the large-mouthed bass (*Huro salmoides*) at graded temperature levels. *Physiol. Zool.,* **17**: 42–50.

Gasser, H. S., 1931. Nerve activity as modified by temperature changes. *Am. Jour. Physiol.,* **97**: 254–270.

Gasser, H. S., 1933. Axon action potentials in nerve. *Cold Spring Harbor Symp.,* **1**: 138–145.

Glasstone, S., K. J. Laidler and H. Eyring, 1941. The theory of rate processes. McGraw-Hill, New York.

Guenther, A. C. L. G., 1868. Catalogue of fishes of the British Museum, Vol. **7**, London.

Guenther, A. C. L. G., 1880. An introduction to the study of fishes. Adam and Charles Black, Pub., Edinburgh.

Gunn, D. L., 1942. Body temperature in poikilothermal animals. *Biol. Revs.*, **17**: 293–314.

Hathaway, E. S., 1927. Quantitative study of the changes produced by acclimatization in the tolerance of high temperatures by fishes and amphibians. *Bull. U. S. Bur. Fish.*, **43**: 169–192.

Haugaard, N. and L. Irving, 1943. The influence of temperature upon the oxygen consumption of the cunner (*Tautologolabrus adspersus* Walbaum) in summer and in winter. *Jour. Cell. Comp. Physiol.*, **21**: 19–26.

Heckel, J. J. and R. Kner, 1858. Die süsswasserfische der öesterreichischen Monarchie mit Rücksicht auf die angränzenden Länder. Leipzig.

Jordan, D. S., 1905. A guide to the study of fishes. Henry Holt and Co., New York.

Jordan, D. S., 1923. A classification of fishes including families and genera as far as known. Stanford Univ. Press, Stanford, California.

Jordan, D. S. and B. W. Evermann, 1898. The fishes of North and Middle America. Vol. **3**, Bull. U. S. National Museum, No. 47, The Smithsonian Institution, Govt. Printing Office, Washington.

Loeb, J. and H. Wasteneys, 1912. On the adaptation of fish (Fundulus) to higher temperatures. *Jour. Exp. Zool.*, **12**: 543–557.

Parker, G. H., 1939. General anaesthesia by cooling. *Proc. Soc. Exp. Biol. and Med.*, **42**: 186–187.

Peiss, C. N. and J. Field, 1948. A comparison of the influence of 2,4-dinitrophenol on the oxygen consumption of rat brain slices and homogenates. *Jour. Biol. Chem.*, **175**: 49–56.

Shelesnyak, M. C., 1948. The history of the Arctic Research Laboratory. *Arctic*, **1**: 97–106.

Sizer, I. W., 1943. Effects of temperature on enzyme kinetics. *Adv. in Enzymology*, **3**: 35–62.

Snedecor, G. W., 1946. Statistical methods. 4th Ed., Iowa State College Press, Ames.

Suomalainen, P., 1939. Hibernation of the hedgehogs. VI. Serum magnesium and calcium. Artificial hibernation. Also a contribution to chemical physiology of diurnal sleep. *Ann. Acad. Scient. Fennicae*, **53**: 1–68.

Stearn, A. E., 1949. Kinetics of biological reactions with special reference to enzymic processes. *Adv. in Enzymology*, **9**: 25–74.

Tait, J., 1922. The heart of hibernating animals. *Am. Jour. Physiol., Proceedings*, **59**: 467.

Umbreit, W. W., R. H. Burris and J. F. Stauffer, 1945. Manometric techniques and related methods for the study of tissue metabolism. Burgess Co., Minneapolis.

Vernon, H. M., 1899–1900. The death temperature of certain marine organisms. *Jour. Physiol.*, **25**: 131–136.

Wells, N. A., 1935. Variations in the respiratory metabolism of the Pacific killifish *Fundulus parvipinnis* due to size, season, and continued constant temperature. *Physiol. Zool.*, **8**: 318–336.

Wells, N. A., 1935a. The influence of temperature upon the respiratory metabolism of the Pacific killifish, *Fundulus parvipinnis*. *Physiol. Zool.*, **8**: 196–227.

Wilson, P. W., 1949. Kinetics and mechanisms of enzyme reactions. *In* Respiratory Enzymes. Burgess Co., Minneapolis, 16–57.

BODY INSULATION OF SOME ARCTIC AND TROPICAL MAMMALS AND BIRDS *

P. F. SCHOLANDER,[1] VLADIMIR WALTERS,[2] RAYMOND HOCK,[3]
AND LAURENCE IRVING[4]

Introduction

Since the early days of polar exploration it has been known that the arctic in the winter months supports a substantial population of mammals and birds and that these maintain a body temperature that falls within the normal limits found in temperate and tropical forms (Back, 1836; Parry and Lyon, 1825, 1827). All of these warm blooded animals, which include man, may at times be subjected to very low environmental temperatures. The coldest on record are − 68° C. in eastern Siberia and − 65° C. in northwestern Canada, with monthly averages running as low as − 60° C. and below − 50° C., respectively (Bartholomew and Herbertson, 1899; Court. Sissenwine and Mitchell, 1949).

This means for some animals the maintenance, for weeks, of a temperature gradient which may be as much as 100° between the interior of the body and the environment. Thermally this difference is equivalent to maintaining the animals at the boiling point of water in a zero degree environment. Both animals and man accomplish this by two principal adjustments: (1) by lowering the heat loss through increasing the insulation, and (2) by increasing the heat production through raising the metabolism. Actually the smaller mammals and birds, together with man, cannot endure the coldest weather continuously but escape, at least for their resting periods, by burrowing into the snow (ptarmigans), or into well insulated nests where they may stay close together (lemmings, mice, etc.), while others may retire under an electric blanket in a well heated hut (explorers). Finally, the hibernators (arctic ground squirrels) spend the winter within insulated burrows at a very low metabolic rate, just sufficient to maintain body temperature above freezing.

In the winters of 1947–48 and 1948–49, a number of species of arctic mammals and birds were captured near the laboratory at Point Barrow (lat. 71° N.), and were kept in outdoor cages in order to permit study of their means of adjustment to cold. At the same time a collection of winter furs was made.

It was considered that much might be gained in understanding adjustment to cold if we could extend our investigation to include tropical mammals and birds. Through the courtesy of the Navy it was possible to undertake such an investigation during three months in Panama at the U. S. Naval Air Station, Coco Solo, Canal

---

* The experimental work was supported through contract with the Office of Naval Research. The preparation of the material for publication was assisted by the Arctic Health Research Center, U. S. Public Health Service.

[1] Now at Department of Biological Chemistry, Harvard Medical School.

[2] Now at Department of Biology, New York University.

[3] Now at Arctic Health Research Center, Anchorage, Alaska.

[4] Now at Arctic Health Research Center, Anchorage, Alaska.

Zone, and three months at the Canal Zone Biological Area (Barro Colorado Island), at latitude 9° N.

In the present paper we shall deal with observations on factors that influence the heat loss from the animal, especially the insulation afforded by the fur and feather covering, as measured in pieces of raw skin and fur taken directly from the skinned animal. The arctic furs were all in prime winter condition. Naturally such measurements can only give a rough estimate of how the body insulation varies from one species to another. The living animal may vary the fur and skin insulation greatly by erecting the hairs, by vasomotor control of the skin and tissue temperature immediately under the fur, by changes in evaporation, etc. There are also considerable regional differences in heat loss of body, legs, and face. Respiration and posture of the animal likewise are major factors in determining the total heat loss. Even the relative figures obtained from such skin patch measurements could be misleading as to the total insulation of the animal. We could easily imagine a chubby arctic animal with short, well insulated legs and tail to have a better overall insulation than a tropical animal with long, poorly insulated arms, legs, and tail, even though the tropical form might have a better body insulation than the arctic form.

In spite of these limitations, it was considered that representative insulation measurements of the furs from a series of mammals from the arctic and the tropics would be indispensable for an analysis of the temperature sensitivity in the living species.

The importance of the natural fur and feather covering as an insulator has been long appreciated (Bergmann, 1847; Rubner, 1924; Lefevre, 1911; Tigerstedt, 1910), but few quantitative data on the subject (Giaja, 1931; Babenyscheff, 1938) have been found.

Material

Raw skins were secured from the following arctic mammals, all caught in the winter except the shrew:

Shrew (*Sorex tundrensis*)
Grizzly bear (*Ursus* sp.)
Polar bear (*Thalarctos maritimus*)
Marten (*Martes americana*)
Least weasel (*Mustela rixosa*)
Red fox (*Vulpes fulva alascensis*)
White fox (*Alopex lagopus*)
Eskimo dog (*Canis familiaris*)
Dall sheep (*Ovis dalli*)
Wolf (*Canis lupus*)
Seal (*Phoca hispida*)
Ground squirrel (*Citellus parryi*)
Beaver (*Castor canadensis*)
Lemming (*Dicrostonyx groenlandicus rubricatus*)
Lemming (*Lemmus trimucronatus*)
Hare (*Lepus americanus*)
Reindeer (*Rangifer*—domesticated)
Caribou (*Rangifer arcticus*)

Fat and pieces of musculature were removed from the raw hide and the hair side was carefully dried. The flesh side was left naturally moist for the measurements. Except for a thermally insignificant localized fat pad on the rump of the reindeer and caribou, none of the mammals (except the seals) has any significant layer of subcutaneous fat or blubber. Subcutaneous fat is a heavy and poor insulator compared to fur and does not seem to play any role at all in the insulation of terrestrial arctic mammals. In the aquatic seals and whales, however, it is the principal or only insulating material. In the seals the skin with blubber adhering to it was detached from the underlying muscle fascia.

Skins from the following tropical mammals were secured in the Panama Canal Zone:

Opossum (*Didelphis marsupialis*)
Night monkey (*Aotus trivirgatus*)
White-faced monkey (*Cebus capucinus*)
Marmoset (titi) (*Leontocebus geoffroyi*)
Three-toed sloth (*Bradypus griseus* or *ignavus*)
Two-toed sloth (*Choloepus hoffmanni*)
Rabbit (*Sylvilagus gabbi*)
Squirrel (*Sciurus granatensis* or *variegatoides*)
Jungle rat (*Proechimys semispinosus*)
Paca (*Cuniculus paca*)
Agouti (*Dasyprocta punctata*)
Raccoon (*Procyon cancrivorus* or *lotor*)
Coati (*Nasua narica*)
Kinkajou (*Potos flavus* or *Bassaricyon gabbi*)
Collared peccary (*Tayassu angulatus*)
Deer (*Mazama sartorii*)

Patches of raw hide large enough for insulation measurements were cleaned, dried, and shipped with disinfectant to the Point Barrow laboratory where the flesh side was remoistened before the measurement. The skin patches were later sent to the Smithsonian Institution, where they were kindly identified by Dr. David H. Johnson and Dr. H. W. Setzer.

INSULATION MEASUREMENT

The heat transmission through the fur was measured by employing a conventional hot plate and guard ring technique. The hot plate was made of two circular aluminum discs with an insulated hot wire spiral between them. A similarly constructed guard ring, as wide as the radius of the plate, surrounded the center plate. The plate and guard ring could be independently heated and their temperatures read by thermocouples attached to the metal surfaces. The heating of the guard ring could be controlled with sufficient accuracy by a variac; the center plate was heated from a storage battery and the energy input was measured by an ammeter and a voltmeter.

Three sizes of such hot plate guard ring units were employed with the hot plate, 6, 3, and 1 dm.^2 in area, respectively, depending upon the thickness and size of the skin samples to be measured. Covered with pieces of uniform celotex, the three plates gave a heat transfer reading per unit surface within 3 per cent of each other, and within 5.5 per cent of the figure reported for celotex in the *Handbook of Chemistry and Physics,* 29, p. 1822.

For the measurement, two similar skin pieces (usually from either side of the back on the same animal, or from the backs of two smaller animals) were stretched flat by means of thumbtacks over a circular hole in two plywood frames. The hot plate unit, placed in a flat thin cellophane bag, was sandwiched between the moist flesh sides of the skins and the frames were clamped together (Fig. 1).

To make the measurement, the frame with skins and hot plate unit was hung up vertically in a room thermostabilized at 0° C. ± 1°. The plate and guard ring were heated to 37° C. and held there until the energy input had reached a constant minimum, indicating that heat saturation and equilibrium between heat input and heat loss had been reached. A heavy skin usually required two to three hours before

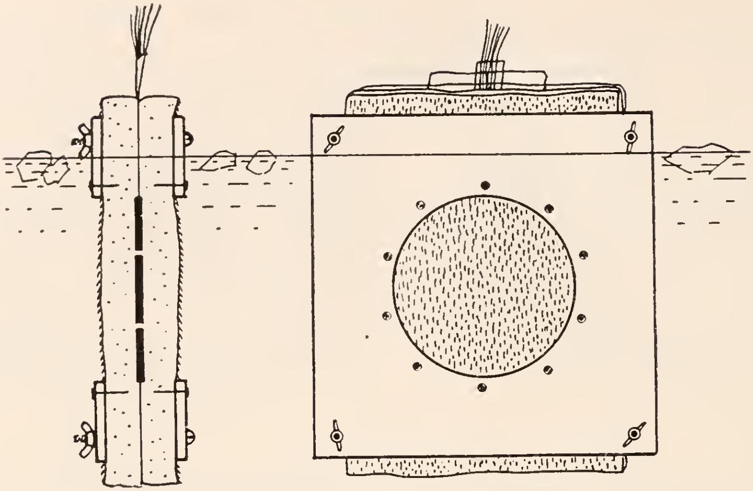


FIGURE 1. Method for measurement of heat transfer through seal skin and blubber in ice water. Left, cross-section through plywood frames holding slabs of seal blubber, clamped around the hot plate guard ring unit. Right, frontal view. Nails are driven through the frame around the center hole to hold the heavy blubber in place. Measurements of the skin of land animals were made with the frame suspended in air at 0° C.

reaching equilibrium. The determinations were made with the fur ruffed up as much as possible for maximum insulating values.

Among arctic animals the insulation of the fur is so large that the hide comprises a relatively small part of the total insulation. The thermal gradients observed in the fur of white fox and caribou showed the surface temperature of the skin to be substantially lower than that of the plate (Fig. 2). This is caused by some ex-

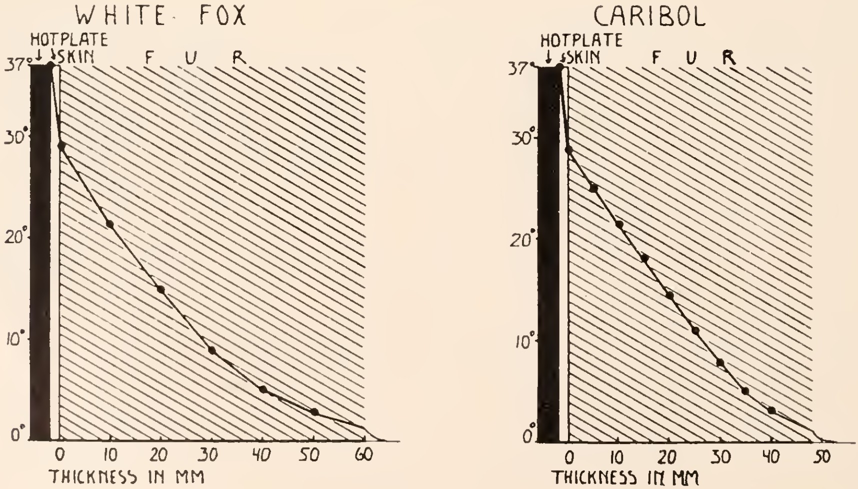


FIGURE 2. Thermal gradients set up in fur of white fox and caribou when in position on the hot plate.

traneous factor, possibly evaporation or poor contact between plate and skin, as it is reasonable to believe that the skin is a poorer insulator than the fur. The decrease in slope of the temperature gradient in the outer layers of the fox fur arises from superficial thinning of the fur. Caribou fur is uniformly dense right to the surface.

In the samples from tropical animals, which often have sparse and short fur, most of the insulation noted in these tests was actually afforded by the hide. This undoubtedly makes the insulation values found for tropical animals considerably higher than in life, when the hide is vascularized, serving heat dissipation rather than heat conservation. In practically naked animals, like the peccary and the paca, the insulation of the fur must be near zero.

Measurement of thickness of the fur is naturally quite arbitrary. A blunt needle was pushed through the fur against the skin and the more or less well defined main surface of the fur set off on the needle with the thumb and measured in millimeters. The average of several such measurements was taken to represent the thickness of the fur.

Insulation in Mammals

The heat transfer was measured in watts per dm.^{2} per 37° C. temperature gradient and the insulation is given as the reciprocal of the heat transfer.[5]

In Figures 3 and 4, observed insulation values are correlated with the thickness of the fur. As was to be expected there is a close correlation with the thickness. None of the furs, under the circumstances tested, were quite as good insulators as an equally thick layer of medium dense cotton. Some of the polar bear furs were conspicuously open and coarse haired and proved to be poor insulators for their thickness.

Among mammals the size of the fox or larger, there is no clear correlation of fur thickness or insulation to the size of the animal (Fig. 5). At the size of the fox, insulation seems to have reached a useful maximum shared by most of the larger forms, including man wearing eskimo parka and pants made of one layer of caribou or other fur. With size decreasing below that of the fox, fur must be shorter and lighter or the animal could not move about. This is particularly true for the smallest forms, the shrew, weasel, and lemming. The ground-squirrels are somewhat out of line, with very little insulation compared to weight. However, they are hardly ever exposed to such gradients as the others, since they escape into their burrows and hibernate. At the lower end of the scale we note that small arctic mammals overlap in insulation value some of the tropical forms, which is a remarkable fact. We shall see, however, that arctic and tropic forms are nevertheless quite different in their temperature sensitivity.

Eskimo dogs and white and red foxes were observed to sleep unprotected on the snow at − 50° C., and so presumably do the wolves, polar bears, caribou, Dall sheep, and other large mammals. The smaller mammals, however, all live in burrows in the winter time and have insulated nests of grass, cotton grass, reindeer hair, etc. Their habits supplement the meager insulation of their fur. As will be shown later, their metabolic heat regulation has already set in before the temperature drops to zero, so that whenever they leave their nests in cold weather they have to be active in order to maintain their body temperature. At Point Barrow the snow temperature at ground level usually stayed at − 25° C. during the winter. One small lemming nest of grass was found in fair condition after the spring thaw and showed an insulating value of the walls, in air, roughly 1.5 times that of the lemming fur. Covered with snow, the insulation could have been no less. Colonial nests, which are much larger, have been observed and it is reasonable to believe that the nests are warm enough to maintain an air temperature around the animals at + 10°

---

[5] Professor Alan C. Burton has kindly informed us that this unit of insulation amounts to about 2.4 Clo units, and that the slope of the fur insulation in Fig. 3 works out at about 3.7 Clo per inch, which is the accepted value for still air in clothing. An arctic uniform has about 5 Clo units or about 35 mm. thickness. It will be noted that this is considerably less than what most of the larger arctic mammals wear on their body.

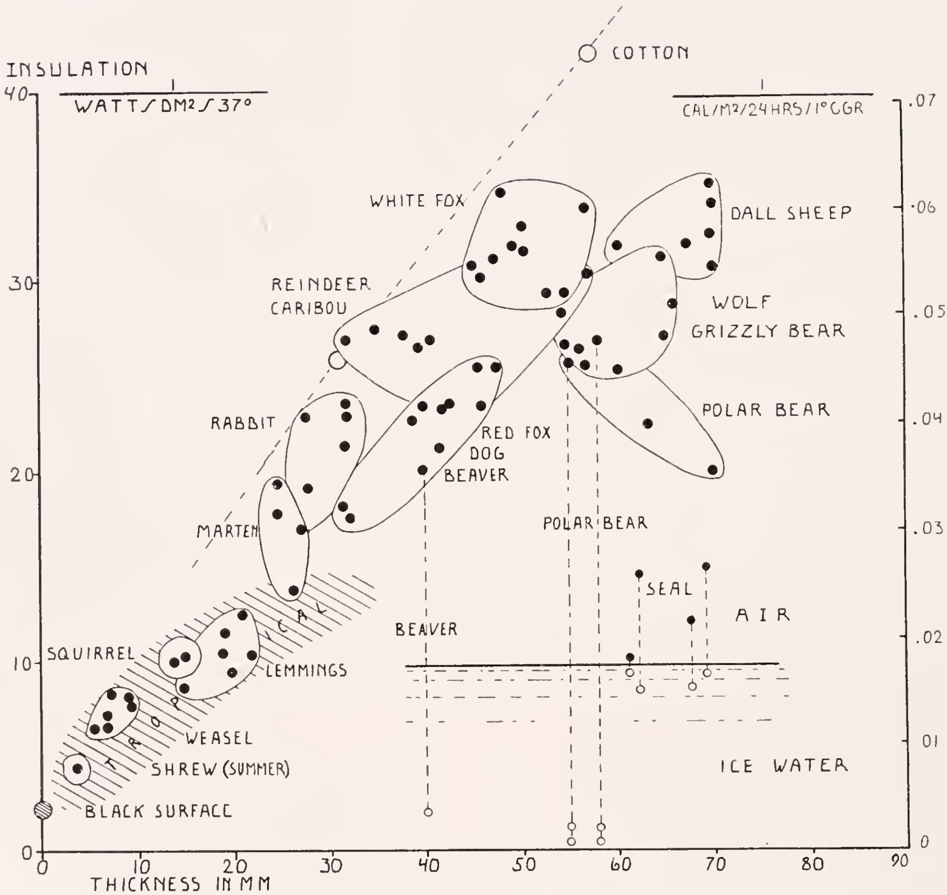


Figure 3. Insulation in relation to winter fur thickness in a series of arctic mammals. The insulation in tropical mammals is indicated by the shaded area. In the aquatic mammals (seal, beaver, polar bear) the measurements in 0° C. air are connected by vertical broken lines with the same measurements taken in ice water. In all cases the hot plate guard ring unit was kept at 37° C. and the outside air or water at 0° C. The two upper points of the lemmings are from *Dicrostonyx*, the others from *Lemmus*.

C. or better, which is their critical temperature. Below 10° C. they would have to raise their metabolic rate.

The shrew, weighing only 1.9 gm., was found in the summer time, and its skin was too small to be measured on the smallest hot plate. The insulation of the summer skin has been estimated from its fur thickness. As in the other animals, its insulation is probably greater in winter.

Among the tropical mammals tested (Fig. 4), the sloths are the best insulated, which seems to be necessitated by their low rate of metabolism. The kinkajou and night monkey, both active nocturnal and arboreal mammals, are relatively well insulated, whereas other nocturnal ground mammals like the paca, opossum, and jungle rat are among the poorest insulated. There does not appear to be any correlation between insulation and diurnal or nocturnal habits. If anything, it would seem that a night sleeper, passive when it is coldest, might need more insulation than a day sleeper. But the microclimates selected are not known. It should not be forgotten that in many boreal, as well as tropical climates, almost naked mammals live side by side with relatively furry mammals, pointing to the potency of peripheral vasomotor control for the regulation of the body insulation.

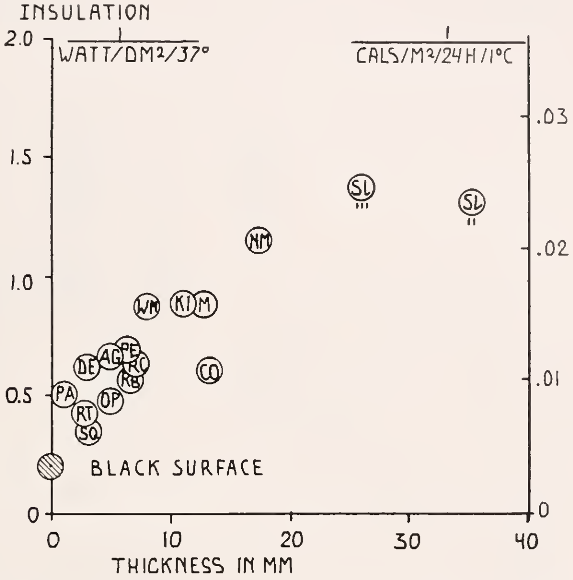


FIGURE 4. Insulation in relation to fur thickness in tropical mammals. Hot plate guard ring unit 37° C., outside air 0° C. The insulation of the fur of most of these animals is probably close to that of a bare black surface but is higher in our measurements because of the relative high insulation of the skin in our dead samples.

AG—agouti
CO—coati
DE—deer
KI—kinkajou
M—marmoset
NM—night monkey
OP—opossum
PA—paca
PE—peccary
RB—rabbit
RC—raccoon
RT—rat
SL II—2-toed cloth
SL III—3-toed sloth
SQ—squirrel
WM—white-faced monkey

Among the smaller arctic mammals like the lemming, weasel, and snow-shoe rabbit, the legs and feet have a considerable fur protection as compared with their southern relatives. However, among the birds that will withstand cold winters, naked legs are most common, and legs insulated with feathers, as in the ptarmigan and snowy owl, are exceptions. In general the insulation on the legs and feet in mammals and birds is very much less than on the body. Several measurements on mountain sheep, caribou, and reindeer showed only from ⅕ to ⅓ as much insulation on the legs as on the body. We know that aquatic birds can stand low leg temperatures. Undoubtedly seals, muskrats, beavers, moose, and other semi-aquatic mammals can also stand low leg temperatures for a long time, as they virtually lack insulation against the ice water. It seems very probable that arctic nonaquatic mammals and birds can also stand prolonged low leg temperatures because the leg insulation is so slight. This assumption is strongly supported by measurements of the melting point of fats taken from caribou legs. Thus Abrahamsen (1950) found that fats

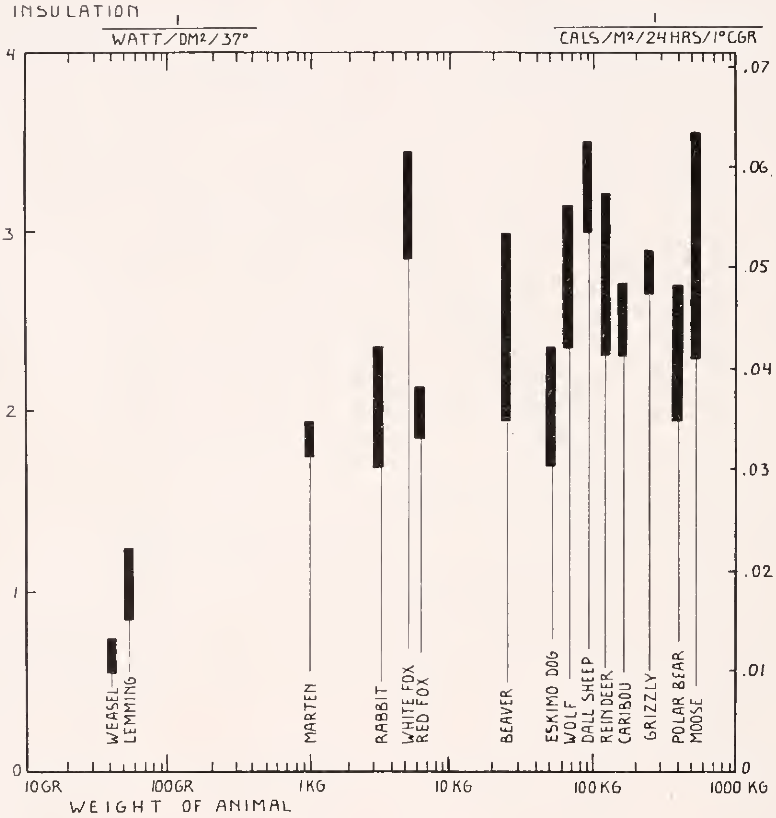


FIGURE 5. Insulation of the winter fur in relation to the body weight in a series of arctic mammals. From a 5 kg. fox to a 500 kg. moose the insulation does not vary much in most of the animals.

taken from the lower parts of the legs had a melting point thirty degrees lower than that of fats from the upper parts of the legs. If poorly circulated the legs would cool and further heat loss would be retarded. Fully circulated, the legs would become warm and would greatly facilitate heat loss on account of their poor insulation. This may, in part, explain the fact that a resting, heavily insulated arctic mammal or bird is nevertheless able to change its heat dissipation by a factor of eleven times. By pilo motor reflex alone it seems doubtful whether a caribou or fox could even double its insulation. The fur of a lemming fluffed up to the maximum, provided roughly only twice as much insulation as the fur with the hairs smoothed down.

Insulation in Birds

Insulation measurements were made on snow bunting and ptarmigan skins. The snow buntings gave figures comparable to those of the lemmings and the ptarmigan figures were comparable to those of the marten. However, these measurements are less satisfactory since it is impossible to produce on the test plate the well-ordered elevation of the feathers which the live bird can achieve to produce maximum insulation.

Our snow buntings spent the winter standing on the board floor in their outdoor wire cage with temperatures often as low as − 40° C. Only in the coldest weather did these birds, ravens and gulls, find it necessary to protect their naked feet by sitting on them. Only a few of the buntings showed evidence of frostbite on their slender naked feet. A dozen arctic gulls walked about all winter on the snow in an outdoor wire cage with temperatures sometimes as low as − 50° C. without freezing their large-surfaced naked webbed feet. A few determinations of the heat dissipation from a live gull's feet in ice water showed it to be very low. It corresponded to only a few cc. of blood an hour, indicating that the temperature of the foot and leg must have been close to zero. Such a low leg temperature can evidently be tolerated for hours at a time and results in general heat conservation for the bird. None of our gulls froze their feet. The circulation must have increased with the increasing gradient so as to keep the feet and legs at just above freezing; and it is likely that the heat loss from the legs and feet, below freezing, increases linearly with the gradient. A gull that had been kept indoors for several months, in a cage usually warmer than 20° C., was observed to freeze the web on its feet, white and hard, in less than one minute after it escaped through an open door onto the snow outside at − 20° C. The outer web and parts of some toes subsequently became gangrenous and were lost. Evidently it had lost its cold adaptation or its vaso motor response was not quick enough.

Insulation Measurements in Aquatic Mammals

In man even a few minutes of partial or total immersion in ice water causes unbearable chill and pain, and yet some arctic mammals spend all or parts of their lives swimming about in icy, subzero waters. It is, therefore, of interest to know something about their body insulation in ice water as compared with that in air. Insulation measurements in ice water were made on skins from the polar bear, beaver, and seal (*Phoca hispida*). This species of seal spends most of its life in waters around 0° C. and possesses only a thin hair covering, but it has a thick layer of blubber

(Fig. 6). The blubber is only sparsely vascularized. When the seal submerges the water penetrates the fur completely, leaving no insulating air layer between the skin and the water.

In a series of experiments the heat transmission through the skin and blubber, when submerged in ice water, was measured (Fig. 3). Similarly, the heat losses in ice water through polar bear skin and beaver skin were measured. It can be seen that seal fur and blubber is not a good insulator in air, only slightly better than that of a lemming, although more than three times as thick. The effectiveness of blubber as an insulator in water is demonstrated by the fact that in one of the seal specimens tested, the insulation in ice water was only 5 per cent less than in zero degree air, which means that the temperature gradient in air and in ice water was taken up almost completely within the blubber, leaving the skin surface correspondingly cool in either medium. In all observations the insulation in ice water was about the same, and the difference from the insulation in air can most likely be ascribed to differences in the fur cover. In a living seal the insulation of the skin

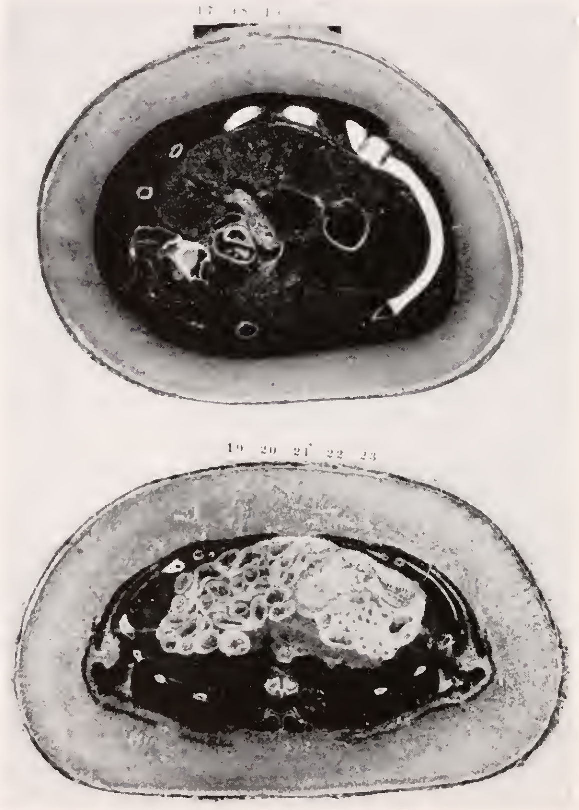


FIGURE 6. Cross sections of two frozen seals (*Phoca hispida*) shot in March, 1948, showing the thick layer of blubber. Tape measure in inches.

and blubber is undoubtedly even less than in our samples because of some subcutaneous and cutaneous circulation. It must be remembered, however, that as long as the seal is in water the temperature gradient is always moderate, never over 40°. Furthermore, the seal is a bulky animal with a high metabolic rate (Irving, Solandt, Solandt and Fisher, 1935; Scholander, 1940). It has in its flippers a highly effective vasomotor control (Scholander, 1940; Irving, Scholander and Grinnell, 1941), and it can readily be seen how even a slight increase in the peripheral circulation could remove large quantities of heat generated by swimming.

In contrast to the seal, the heat loss through polar bear skin increases 20–25 times when submerged in quiet ice water, and 45–50 times when the water is agitated. The ice water penetrates immediately to the skin surface, dislodging all air, and there is no subcutaneous blubber to afford insulation. Undoubtedly, therefore, the polar bear upon immersion suffers a considerable heat loss at the skin surface. This is probably reduced by peripheral vasoconstriction and cooling and can apparently be compensated for over a long time by heat production during swimming. The coarse and open polar bear fur sheds water very easily upon shaking, so that the insulation is readily restored when the bear emerges. The polar bear is also a very large animal with large heat capacity and has a proportionately small surface.

In contrast to the seal and polar bear, the beaver has an extraordinarily dense and fine fur which retains a layer of air several millimeters thick next to the skin when submerged. This can be easily observed on a submerged skin sample. It undoubtedly helps the insulation, which nevertheless is surprisingly low in ice water.

SUMMARY

Insulation measurements on raw skins from 16 arctic and 16 tropical mammals are given. There is, as would be expected, a good correlation between the thickness of the fur and the insulation. The smaller arctic mammals (weasels, lemmings) have much less insulation than the larger and overlap many of the tropical forms. From the size of a fox to the size of a moose there is no correlation between insulation and body size, they all have about the same insulation per surface area. When submerged in ice water, seal blubber retains about the same good insulation, as compared with measurements taken in 0° C. air. In the polar bear, heat transfer through the fur increases 25–50 times when submerged, because of complete wetting of the skin surface and absence of blubber. The beaver is slightly better off when submerged, as it retains an insulating layer of air in the fur next to the skin.

ACKNOWLEDGMENTS

We are greatly indebted to the officers, civilians, and natives of the Barrow camp for the many efforts that they expended in our behalf. We wish to express our indebtedness to Captain H. W. Taylor, USN, Commanding Officer at the U. S. Naval Air Station, Coco Solo, Canal Zone, for facilitating our work there in every way. We also wish to acknowledge with gratitude the great help given us by Dr. James Zetek, U. S. Department of Agriculture, Balboa, Canal Zone. We are much indebted to Dr. David H. Johnson and Dr. H. W. Setzer at the Smithsonian Institution for kindly identifying the scant skin fragments of the tropical animals, and to Mr. Robert H. Stapleton and Mr. Walter B. Flagg for assistance in many ways. Dr. R. Wennesland rendered valuable assistance in the insulation measurements of arctic furs. We are greatly indebted to Dr. Alfred C. Redfield, Dr. Cecil K. Drinker and Dr. Alan C. Burton for inspiring discussions and criticism.

LITERATURE CITED

Abrahamsen, N., 1950. Unpublished.

Babenyscheff, V. P., 1938. Heat loss of different species of rodents in relation to the heat conduction of their fur and the size of their body and the resistance as regards climatic factors. *Zoologitsheskij Jour.*, **17**: 540.

Back, 1836. Température des animaux par de très grands froids. *Compt. Rendus Acad. Sci., Paris,* **2**: 621.

Bartholomew, J. G. and A. J. Herbertson, 1899. Physical atlas. Volume III. Roy. Geogr. Soc., Westminster.

Bergmann, A., 1847. Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. *Göttinger Studien*, **1**: 595.

Court, A., N. Sissenwine and G. S. Mitchell, 1949. Lowest temperatures in the Northern Hemisphere. *Weatherwise*, **2**: 1.

Giaja, A., 1931. Contribution à l'étude de la thermorégulation des oiseaux. *Ann. Physiol. Physicochim. Biol.,* **7**: 13.

Handbook of chemistry and physics, 1947. **29**: 1822. Cleveland.

Irving, Laurence, P. F. Scholander and S. W. Grinnell, 1941. Significance of the heart rate to the diving ability of seals. *Jour. Cell. Comp. Physiol.,* **18**: 283.

Irving, Laurence, O. M. Solandt, D. Y. Solandt and K. C. Fisher, 1935. The respiratory metabolism of the seal and its adjustment to diving. *Jour. Cell. Comp. Physiol.,* **7**: 137.

Lefèvre, J., 1911. Chaleur animale et bioenergétique. Paris.

Parry and Lyon, 1825. *Ann. Chim. Phys.* **28**: 223.

Parry and Lyon, 1827. *Ann. Chim. Phys.,* **34**: 66. (Quoted by Tigerstedt, 1910.)

Rubner, M., 1924. Über die Bildung der Körpermasse im Tierreich und die Beziehung der Masse zum Energieverbrauch. *Sitzber. Preuss. Akad. Wiss.,* **17**: 217.

Scholander, P. F., 1940. Experimental investigations on the respiratory function in diving mammals and birds. *Hvalraadets Skr.,* **22**: Det Norske Vidensk.-Akad. Oslo.

Tigerstedt, R., 1910. Die Produktion von Wärme und der Wärmehaushalt. *In* H. Winterstein, Handbuch der Vergleichenden Physiologie. Jena.

HEAT REGULATION IN SOME ARCTIC AND TROPICAL MAMMALS AND BIRDS *

P. F. SCHOLANDER,[1] RAYMOND HOCK,[2] VLADIMIR WALTERS,[3]
FRED JOHNSON,[4] AND LAURENCE IRVING[5]

In the preceding paper are presented observations and measurements of the body insulation of a series of arctic and tropical mammals and birds. In the present paper we shall describe experiments in which our animals were subjected to gradually lowered air temperatures in a respiration chamber, where the heat production was simultaneously determined through the oxygen consumption or carbon dioxide production. Similar experiments have been conducted earlier on many laboratory mammals and birds and a few wild animals. Excellent data were obtained by Theodor (1878), Falloise (1900), Rubner (1902), Martin (1902), Goto (1923), Terroine and Trautmann (1927), Benedict and MacLeod (1929), Giaja (1931), Kayser (1930, 1937), Benedict and Fox (1933), Gelineo (1934), Swift and Forbes (1939), Kendeigh (1939, 1944), Kalabukhov (1940), and Herrington (1940).

From the earliest investigations it was made clear that down to a certain environmental temperature (the critical temperature) the body temperature could be maintained, without increasing the metabolism, simply by increasing the insulation (so-called physical heat regulation). Below the critical temperature the body temperature could be maintained only by increasing the heat production (so-called metabolic or "chemical" heat regulation). For geese and rabbits it was found that the critical temperature could be as low as 0° C., and from observed fact that eskimo dogs sleep on the snow at − 40° C. or below we could expect to find arctic mammals having a critical temperature that low or even lower. It was considered likely that cold experiments on tropical mammals and birds would furnish a valuable contrast to our arctic material, and through the courtesy of the Navy we were granted a six months stay in the Panama Canal Zone for the study of this and other problems.

Animal Material

The following arctic mammals were used in our experiments at Point Barrow, Alaska, latitude 71° N.:

White fox (*Alopex lagopus*)
Eskimo dog (*Canis familiaris*)
Polar bear cubs (*Thalarctos maritimus*)
Ground squirrel (*Citellus parryii*)
Lemming (*Dicrostonyx groenlandicus rubricatus*)
Weasel (*Mustela rixosa*)

---

* The experimental work was supported through contract with the Office of Naval Research. The preparation of the material for publication was assisted by the Arctic Health Research Center, U. S. Public Health Service.

[1] Now at Department of Biological Chemistry, Harvard Medical School.

[2] Now at Arctic Health Research Center, Anchorage, Alaska.

[3] Now at Department of Biology, New York University.

[4] Now at Department of Zoology, Cornell University.

[5] Now at Arctic Health Research Center, Anchorage, Alaska.

and the following birds:

Arctic gull (*Larus hyperboreus*)
Canada jay (*Perisoreus canadensis*)
Snow bunting (*Plectrophenax nivalis*)

The dogs, foxes, and polar bear cubs were kept in outdoor wire cages at an average temperature of − 25° C. The weasels, lemmings, and ground squirrels were kept in an animal house at around − 10° C. to + 5° C. These small animals all built nests into which they could retire and in which they spent most of their time. The gulls and snow buntings were caught near the laboratory in September and were kept outside in wire cages. Normally they migrate south during the winter. The jays were caught 200 miles south of the laboratory in April. All of the animals outdoors had access to indoor nest boxes through openings in the wall of the animal house; these, however, they seldom used, even at temperatures − 40° C. or below.

The tropical mammals and birds used for our cold experiments were all caught in the Canal Zone (Panama) and were kept in wire cages at the Barro Colorado Island laboratory (latitude 9° N.) where the outdoor temperature is usually 25° C to 30° C.

The following tropical mammals were obtained and used:

Two-toed sloth (*Choloepus hoffmanni*)
Night monkey (*Aotus trivirgatus*)
Marmoset (titi) (*Leontocebus geoffroyi*)
Raccoon (*Procyon cancrivorus*)
Coati (*Nasua narica*)
Jungle rat (*Proechimys semispinosus*)

and the following tropical birds:

Manakin (*Pipra mentalis*)
Night hawk (Paurangue) (*Nyctidromus albicollis*)

EXPERIMENTAL

In order to be able to compare the effect of temperature on the metabolism in widely different mammals and birds we have referred to the average metabolic level at rest as 100, determined as O_2 consumption or CO_2 production in the thermoneutral zone (= temperature zone of lowest metabolism). All changes are given in relation to that figure.

The arctic animals were studied during the winter of 1947–1948 in an interrupted sequence for each animal, in such a way, however, that each animal covered the whole range of temperatures. The basal reference level of heat expenditure could therefore be established empirically for each individual, which eliminated the uncertainty involved in correcting the metabolism for differences in weight. The animals were given a one to two hour period in the chamber at the proper temperature before a series of determinations was made, extending over another period of one to three hours.

The tropical mammals and birds could withstand only a relatively small range of external temperatures and each individual could therefore be run through the whole temperature range in a single day, starting at room temperature and thereafter going down step by step. The animal usually remained in the respiration chamber for eight to twelve hours, with about two hours at each temperature and allowing one hour for bringing the temperature down.

APPARATUS

A wooden box was mounted outside one of the windows of the laboratory at Point Barrow. The box was furnished with a window facing the laboratory window and was equipped with a fan and a resistance controlled electric heater. The box could be maintained at any temperature warmer than the outside air. The animal was placed on top of a narrow piece of plywood in a wire screen cage just large enough to hold it without cramping. The cage was placed on a pan furnished with a narrow well containing a non-freezing glycerine solution. Over the cage was put a closely fitting sheet metal respiration chamber resting with its lower edge in the glycerine well to form an airtight seal. The chamber carried tygon tubes for in- and outgoing air and a thermometer. The chamber air was adjusted to the proper temperature by regulating the box temperature. It is realized that the air temperature in a small respiration chamber is not an ideal way of characterizing the environmental heat situation. However, it is comparable to all other similar experiments.[6] Heat loss by evaporation was always low because of high relative humidity in the small respiration chamber.

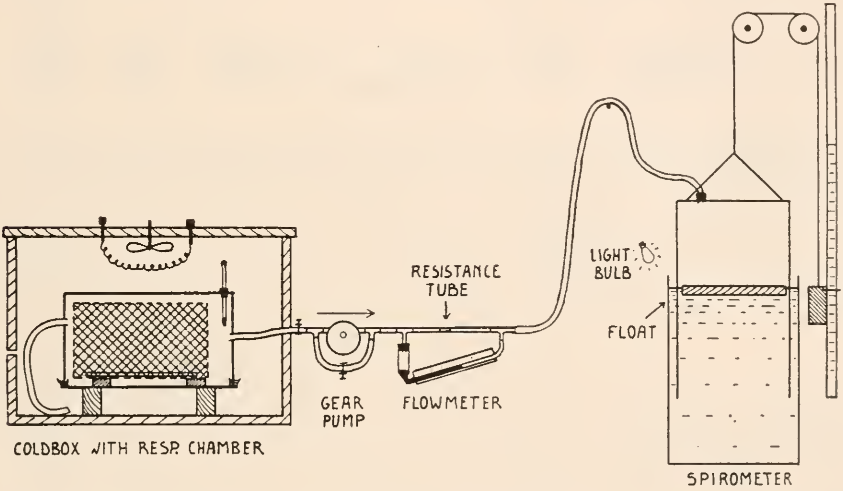


FIGURE I. Open circuit metabolism apparatus used in the cold experiments. A wooden float covered with sheet metal protects the water from gas diffusion. The light bulb keeps the spirometer gases well stirred by setting up convection currents.

Outdoor air, prewarmed in the wooden box, entered the respiration chamber through a six-foot length of tubing. Air was exhausted from the chamber at the other end by means of a small gear pump which propelled it through a flow meter into a respirometer. The speed of the gear pump and the shunt was adjusted so as to give a ventilation through the chamber which would maintain the carbon dioxide of the exhaust air at about 1 per cent, as checked by gas analysis. As our animals varied greatly in weight and metabolic rates, we needed three sizes of spirometers and respiration chambers, two sizes of gear pumps and different bore plastic resistance tubes in the flow meter in order to maintain the same degree of accuracy throughout the series.

---

[6] In his classical work, Martin (1902) gives only the waterbath temperatures. The air temperature in his glass respiration chamber might have been considerably warmer.

Small mammals or birds were usually placed on a narrow piece of plywood in two small tin cans taped together at the openings and furnished with the necessary tubes for in- and outgoing air and temperature measurements. The ventilation was measured on the spirometer and samples were taken out for analysis using either the ½ cc. analyzer (Scholander, 1947) for CO_2 and O_2, or in Panama, the Haldane apparatus for CO_2. A light bulb on one side of the spirometer kept the gases well

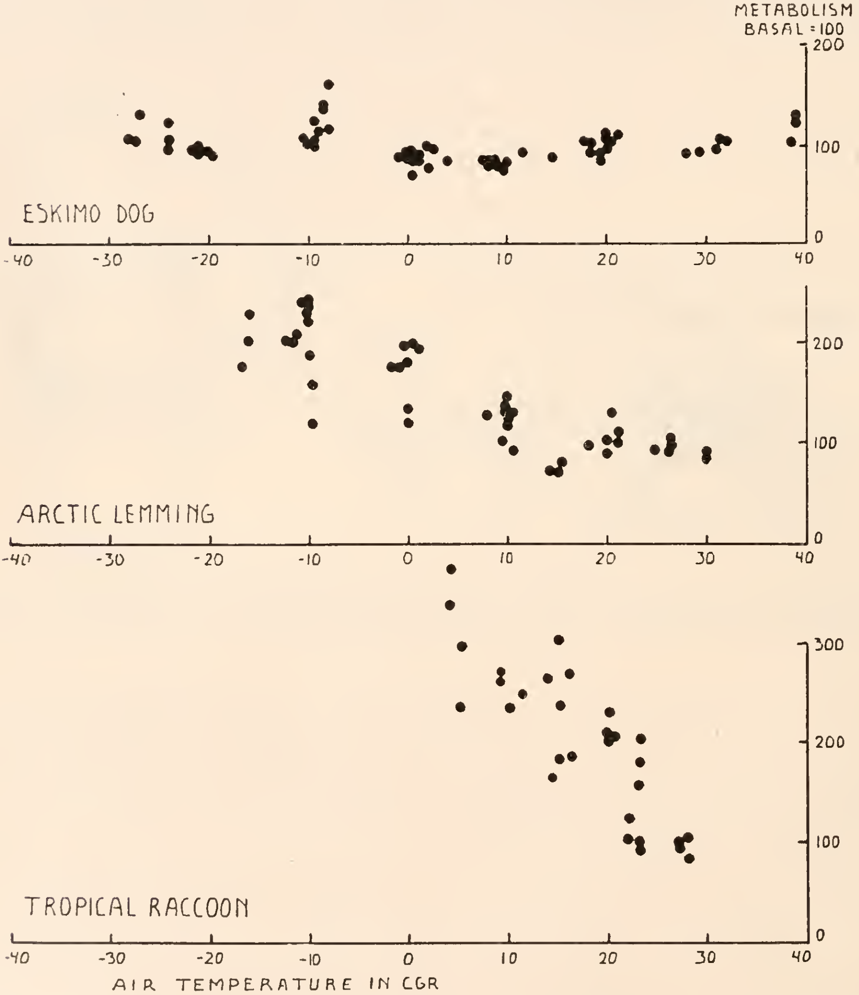


FIGURE 2. Effect of environmental temperature on the metabolic rate in eskimo dog, arctic lemming, and tropical raccoon expressed in terms of basal metabolic rate = 100. The steepness of the curves describes the relative temperature sensitivity of the animal but does not correlate with the weight of the animals or with their body insulation.

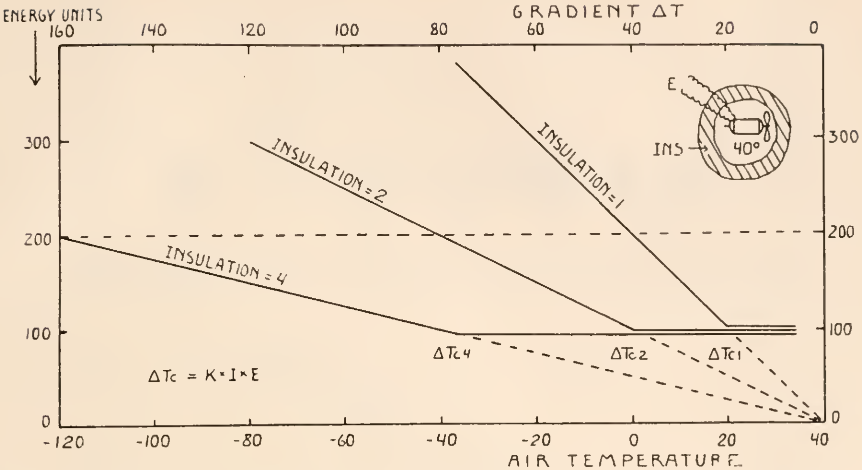


FIGURE 3. Diagram of a thermoregulated system consisting of an electric fan inside a rigid insulator (insert). The full drawn lines represent the relative energy input necessary to maintain the "body temperature" of 40° C. for different air temperatures, assuming a minimum (basal) energy level of 100 and a maximum insulation of 1, 2, and 4 units. The thermoregulation is assumed to proceed in two steps: first an increase in insulation until it reaches maximum at the critical temperature, then an increase in the metabolic rate. At the critical temperature the body-to-air gradient is called the critical gradient ΔTC. At constant insulation the system cools proportionally to the gradient and it will be seen from the figure that the critical gradient is proportional to both insulation and metabolic rate according to the equation given.

stirred by setting up slight convection currents in the spirometer. The accuracy obtained by this well established open circuit technique was ample for our purpose.

For cooling the tropical animals, a deep freeze box was used with an insulated wooden lid furnished with fan and heater. In this was placed the respiration chamber as already described.

The rate of oxygen consumption (arctic animals) or CO_2 production (tropical animals) relative to temperature was plotted for each individual animal, the basal level taken from the plot, and the data recalculated and replotted relative to the basal level which is called 100 (Figs. 2–11).

In the large series of arctic experiments performed by Hock, both CO_2 production and oxygen consumption were determined. The RQ was rarely outside 0.70–0.85 all through the temperature ranges, and hence either oxygen consumption or CO_2 production can be taken as proportional to the heat production, and either one can accurately enough serve our purpose to describe relative changes in heat production.

THEORETICAL CONSIDERATIONS

It became clear from the very beginning of these experiments that the larger arctic mammals like the fox and dog did not need to increase their heat production to stand the relatively modest temperature of − 30° C. that we were able to offer them, and that their metabolism remained essentially unchanged from + 30° C. to − 30° C. However, the smaller arctic mammals and birds soon reacted by slowly increasing their metabolism with falling temperature.

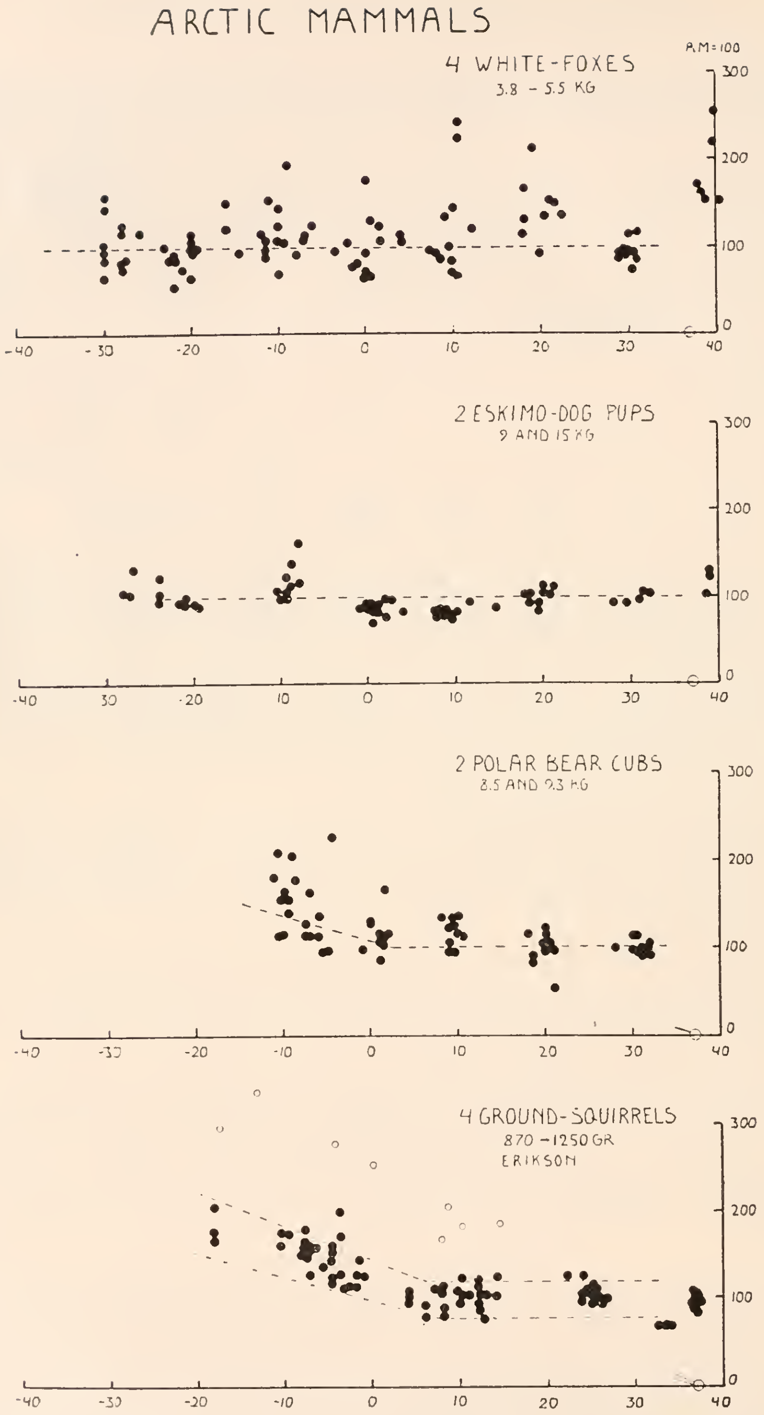


FIGURE 4. Metabolic heat regulation in arctic mammals. In the foxes and husky pups thermoneutrality extends from + 30° C. to at least − 30° C. The open circles in the ground squirrel plot (Erikson, 1950) indicate very active runs where the animal tried to escape.

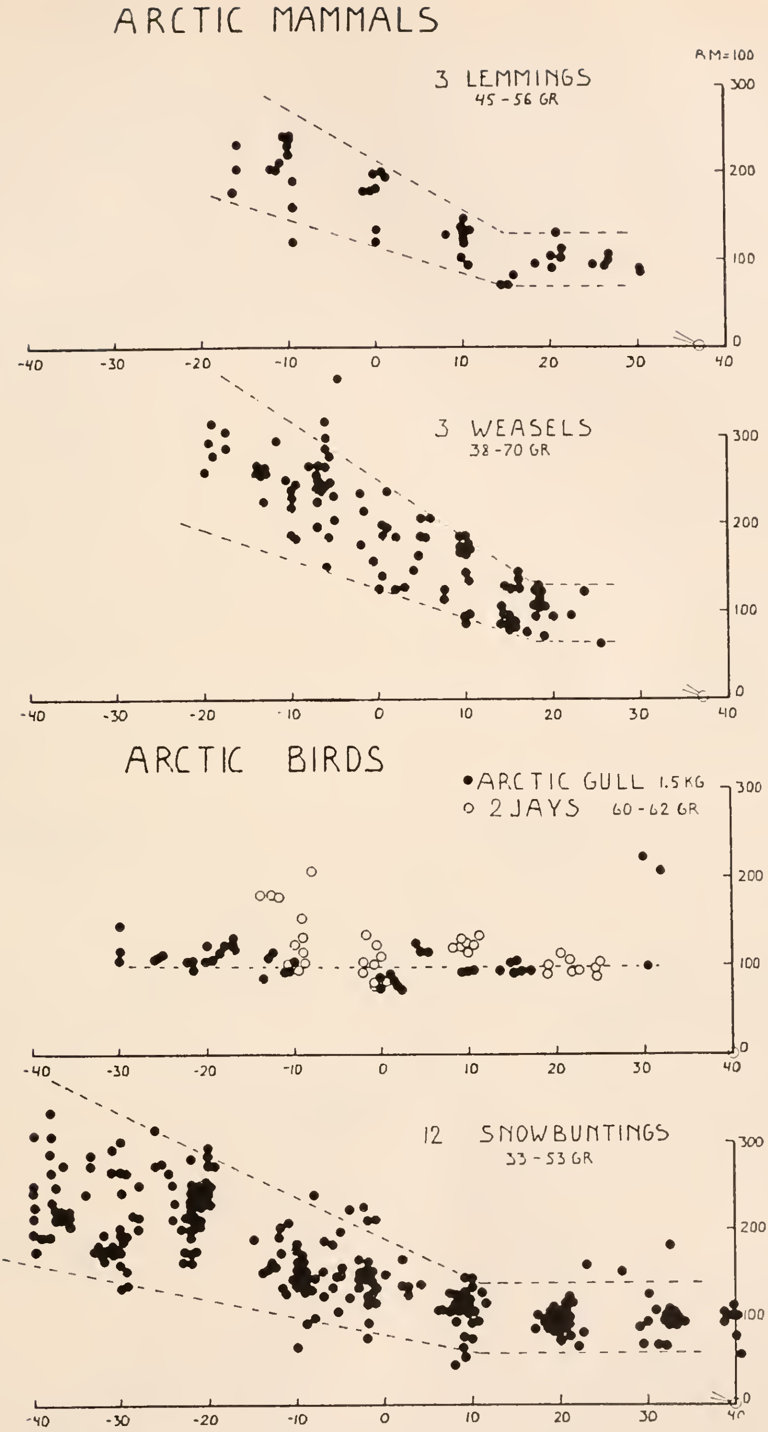
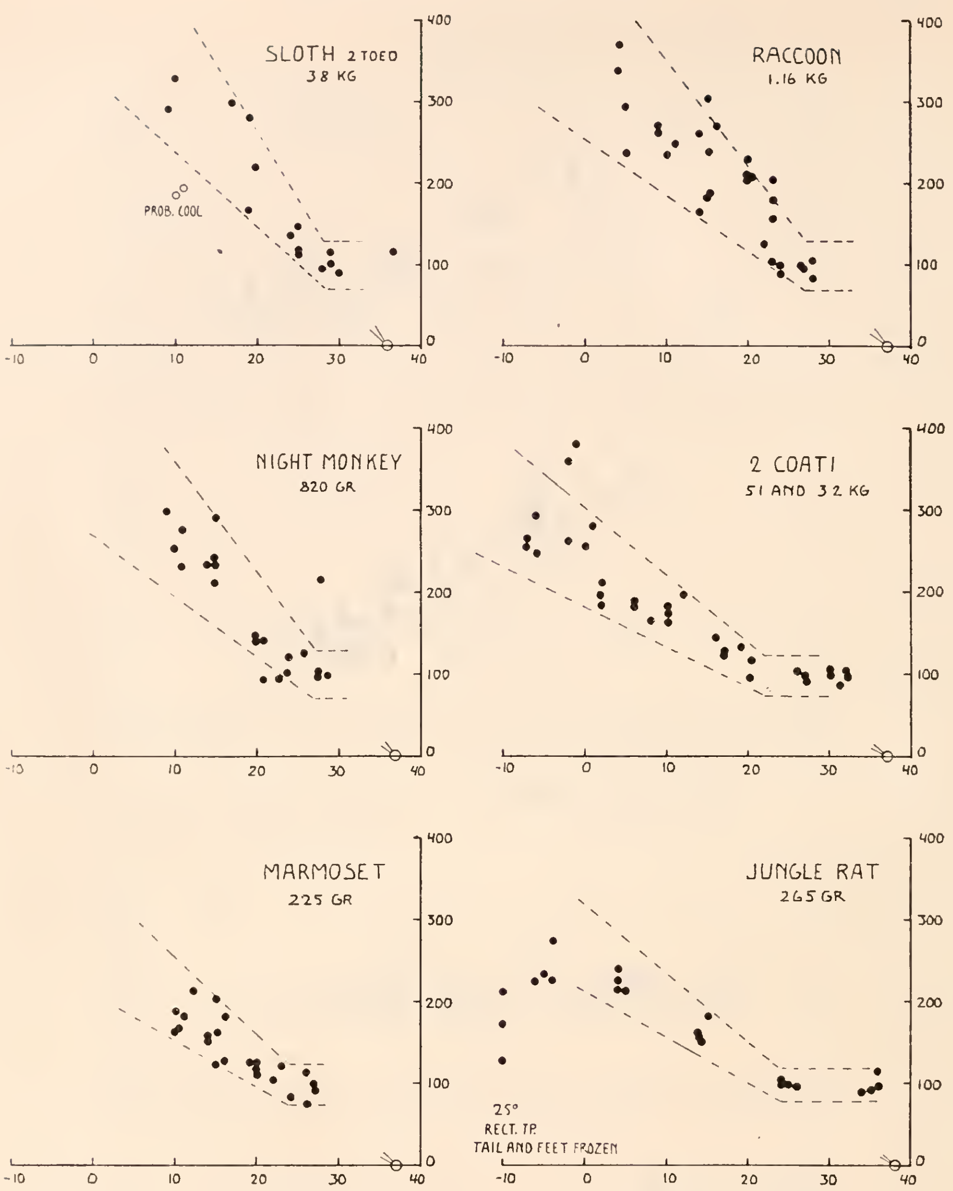
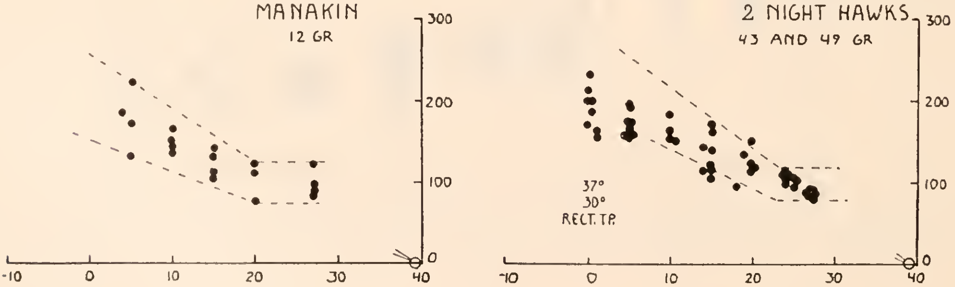


FIGURE 5. Metabolic heat regulation in small arctic mammals and in arctic birds.

TROPICAL MAMMALS



TROPICAL BIRDS



In Panama, it was established that the tropical mammals and birds responded with an increase in metabolism starting at only a few degrees below the ambient air temperature, producing strikingly steep curves compared with those of the arctic animals (Fig. 2). Such differences in slope had been observed by Lapicque (1921) in different animals, and the curves were considered by him to flatten out with increase in size of the animal (Brody, 1945). The relation to size does not hold for the three animals given in Figure 2, however, for the steep-curved tropical raccoon is many times heavier than the little flat-curved arctic lemming. It might be assumed that the curves would become less steep with increasing insulation. However, the tropical raccoon has about the same insulation as the arctic lemming but has a much steeper curve. Altogether contrary to the assumption that insulation alone determines the slope, is the fact that a sloth has a much steeper curve than the less well insulated arctic weasel (Figs. 5 and 6).

Some simple physical considerations lead to an explanation which fits the facts surprisingly well and which also explains, for instance, how an arctic animal can endure temperatures that occasionally may drop as low as − 70° C. A physical analogy or model is useful to clarify what seem to be the main points.

Let us consider a warm-blooded animal as a unit capable of maintaining a constant body temperature in a changing environmental temperature, and that it can do this by adjusting (1) its insulation and (2) its metabolism. The major part of the metabolic increase which a mammal or bird can mobilize against external cooling results from muscular movements in the form of shivering and gross activity. We shall, therefore, picture our warm-blooded animal as an electric motor enclosed in an insulated bag. We shall assume a constant surface area and configuration of the bag and we shall maintain the interior of the bag at body temperature, say 40° C., by regulating the energy input to the motor. We are only studying situations of steady-state heat equilibrium where rate of heat loss equals rate of heat production. It has long been known that the overall heat loss of such a system in a uniform air current is proportional to the temperature gradient between the inside and the outside, according to Newton's law of cooling (Mitchell, 1901). That this also holds true in still air with considerable accuracy is evident from experiments by Burton, 1934 (16° gradient), and by Winslow, Gagge, Greenburg, Moriyama and Rodee, 1935 (27° gradient), and from numerous experiments with heated clothing dummies (copper man). According to the radiation law of Stefan (McAdams, 1942) we should expect, with increasing gradients, progressively smaller values than those predicted from Newton's law. However, as the surface of a good insulator (such as the fur in our arctic animals) progressively cools with the environmental temperature, we are mostly dealing with surface to air gradients which hardly exceed 10–20° and the calculated discrepancy for such small gradients is negligible, especially when compared with the great variation found in our animal experiments. In the present connection, therefore, Newton's law of overall cooling of physical

FIGURE 6. Metabolic heat regulation in tropical mammals and birds. In most of them there is a fair proportionality to the body-to-air gradient. At the lowest temperatures the metabolism dropped in the jungle rat and the night hawks, as a result of falling body temperature. The two circles in the sloth plot were the last determinations and the animal was undoubtedly cool (no body temperature taken).

bodies can be applied as a natural basis for discussing the overall heat loss of animated bodies at different temperature gradients.[7]

Our heat regulating model is pictured in Figure 3, showing an electric motor (fan) in an insulated bag and a diagram relating the energy input necessary to maintain a constant body temperature for different gradients and insulations.

For a given insulation value (1) of the system the energy input for different temperature gradients (ΔT) would follow the diagonal (Ins. = 1), starting with zero energy at zero gradient. If the total insulation were twice as good, the rate of heat trasfer would be only half and the energy input would consequently be only half, as indicated by the diagonal (Ins. = 2). Similarly with four times as good an insulation the rate of energy input would be one quarter as great as one and would be represented by the diagonal (Ins. = 4).

Now let us assume that our model, like the animals, can regulate its insulation and that it has a basal level below which the energy production does not fall. We shall further assume that the thermoregulation in our model always proceeds according to the following sequence when the external temperature falls: first, a gradual increase in the insulation alone until it has reached its maximum and thereafter a gradual increase in the energy input. In an animal the first part would correspond to: a zone of physical heat regulation, which at the critical temperature would change into the second part: the zone of metabolic (chemical) heat regulation, *i.e.,* we presume a complete separation between the physical and chemical heat regulation. Suppose now that we start with our model in air at + 30° C. As we lower the temperature we increase the insulation correspondingly, running horizontally, until we reach 20° C. air temperature. If the insulation is then at its maximum (Ins. = 1) the curve will break and the energy input will increase proportionally to the gradient according to the diagonal marked Ins. = 1. If, however, the insulation can be steadily increased to a value of 2, the critical temperature will be at 0° C. and the energy input will, from then on, follow the line marked Ins. = 2. Similarly if the insulation can be increased 4 times and no more, the critical temperature will move to − 40° C. and from then on the energy input will again be proportional to the gradient and will follow the diagonal marked Ins. = 4.

We have, according to convention, called the lowest temperature at which our model or animal can maintain body temperature without increasing metabolism, its critical temperature (TC), and we shall designate the corresponding temperature gradient between body and environment as the critical gradient (ΔTC). In other words, critical gradient is the greatest temperature gradient which the physical heat regulation alone can take care of.

It will be seen from the Figure that the critical gradient for any given energy level is directly proportional to the insulation. If a system (Ins. = 1) has a critical gradient of 20° C. and is at 20° C. air temperature, doubling the insulation would double the critical gradient and move the critical temperature to 0° C. If another system (Ins. = 2) has its critical temperature at 0° C. and doubles its insulation, the critical gradient will double and the critical temperature move out to − 40° C. Similarly, if a system with a fixed energy level can maintain a constant body temperature of 37° C. in an environmental temperature that changes from + 30° C. to − 40° C., as an arctic fox or dog can do, then we know that the gradient increased from 7° C. to 77° C. and hence that the insulation also increased by the same amount, namely, 11 times!

---

[7] Dr. Alan C. Burton has kindly informed us that the theoretical drop from linearity by the radiation equation in actuality is counteracted by an increase in convection loss due to increased heat capacity as air gets cooler.

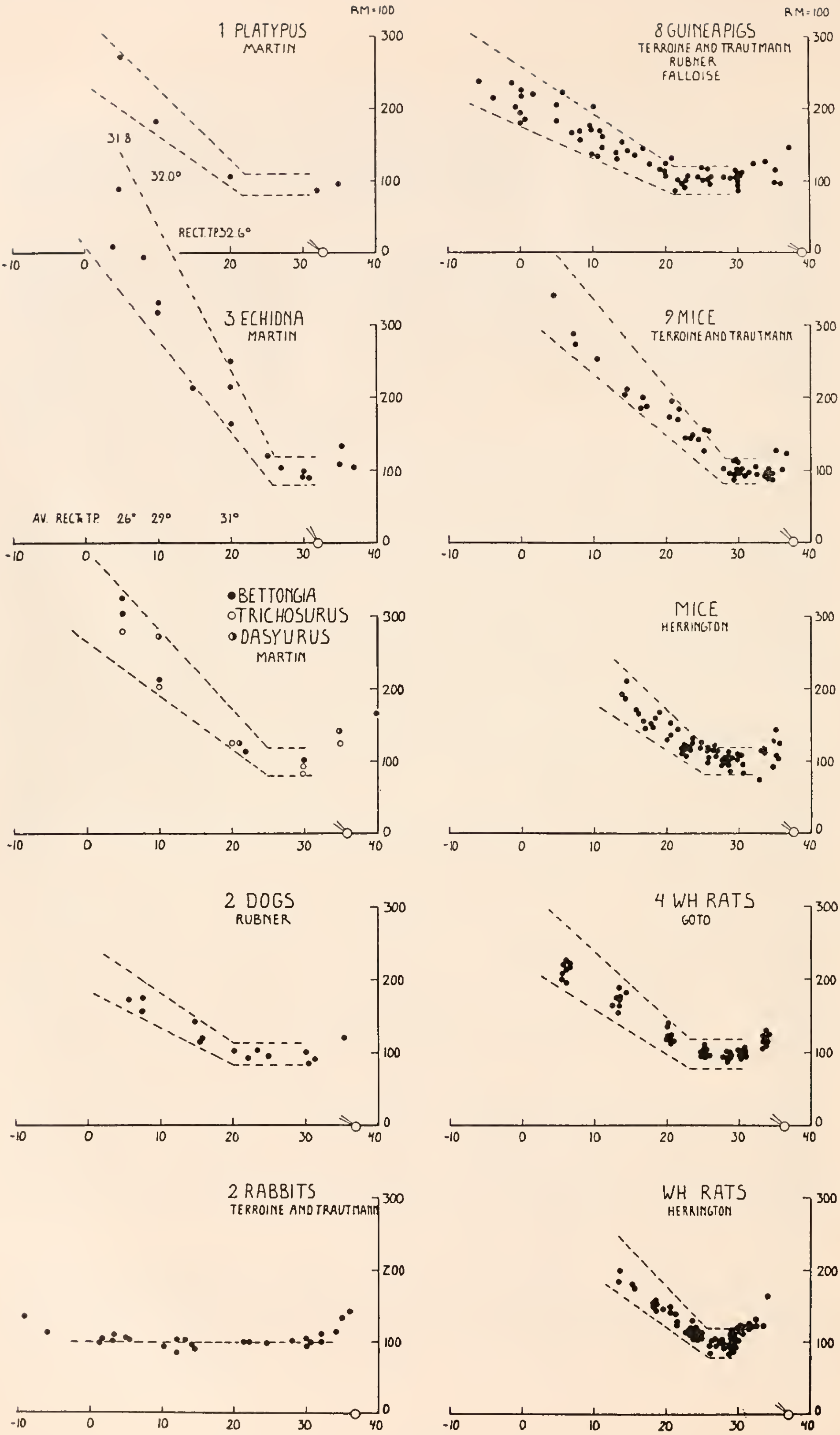
If we now consider the three systems (Ins. = 1, 2, and 4) and we double the energy level in all of them (from 100 to 200) we see that the critical gradient (ΔTC) will also double in each case; in other words, the critical gradient is proportional to the energy level. Physiologically this is of considerable interest. Doubling the metabolism in each system will move the critical temperature of Ins. = 1 from 20° C. to 0° C., of Ins. = 2 from 0° C. to − 40° C., and of Ins. = 4 from − 40° C. to − 120° C.! An arctic fox with body temperature of 40° C. and a critical temperature at − 40° C. can, merely by doubling its metabolism, go to − 120° C., and it takes only a 37 per cent increase in heat production to sustain it in the coldest temperature recorded on earth, somewhere near − 70° C.

The dependence of the critical gradient on the metabolism and the overall insulation can be combined in a simple formula: stating that the critical gradient is proportional to the product of the basal metabolism and the maximal overall insulation, or $\Delta TC = K \times I \times E$, where ΔTC is the critical gradient, K is a constant denoting the units, I is the maximal overall insulation of the system and E is the basal energy level. As we shall see, this explains why a sloth, with a greater fur insulation than the arctic weasel and lemming and our other tropical animals, is still one of the most temperate sensitive animals because of its well known low metabolism.

We have now discussed some fundamental properties of a simple thermoregulated system with only two variables, insulation and metabolism. Our next task is to see to what extent we can expect this analogy to be applicable to a homoiothermic animal. Most authors (Hoesslin, 1888; Lefevre, 1911; Terroine and Zunz, 1925; DuBois, 1936) have emphasized the observation that heat loss from a warm-blooded animal is not proportional to the temperature gradient, and generally speaking this is true. The matter has been somewhat confused, however, by the failure to realize that proportionality should only hold below the critical temperature. Dahr (1926) cites the case of a guinea pig experiment of Rubner's as evidence that they lost heat according to Stefan's law of radiation, but uses body temperature instead of surface temperature. Actually his example nicely illustrates a linear relation to the body gradient. Martin (1902) points out that the heat production of Echidna is proportional to the gradient. Lapicque (1921) and Kleiber and Dougherty (1934) assume, for theoretical reasons, an approximate applicability of Newton's law of cooling below the critical temperature, but do not furnish any factual evidence for it.

To what extent Newton's law would hold below the critical temperature depends upon several factors, the most important being (1) the constancy of the body temperature, (2) the degree of constancy of the overall insulation below the critical temperature, and (3) the degree of constancy of the heat production for each temperature. We shall discuss these factors below.

1. *Body temperature.* Generally speaking, the least variable factor in the whole picture of homoiothermy is the body temperature (Wetmore, 1921; Wislocki, 1933). The poorest temperature regulators are bats, which in the resting state are practically poikilothermous (Hock, 1949). Relatively poor regulators are the mono-


RM=100
1 PLATYPUS
MARTIN
31.8
32.0°
RECT. TP 32.6°
3 ECHIDNA
MARTIN
AV. RECT. TP. 26° 29° 31°
●BETTONGIA
○TRICHOSURUS
⊙DASYURUS
MARTIN
2 DOGS
RUBNER
2 RABBITS
TERROINE AND TRAUTMANN
RM=100
8 GUINEA PIGS
TERROINE AND TRAUTMANN
RUBNER
FALLOISE
9 MICE
TERROINE AND TRAUTMANN
MICE
HERRINGTON
4 WH RATS
GOTO
WH RATS
HERRINGTON
0
100
200
300
-10
0
10
20
30
40

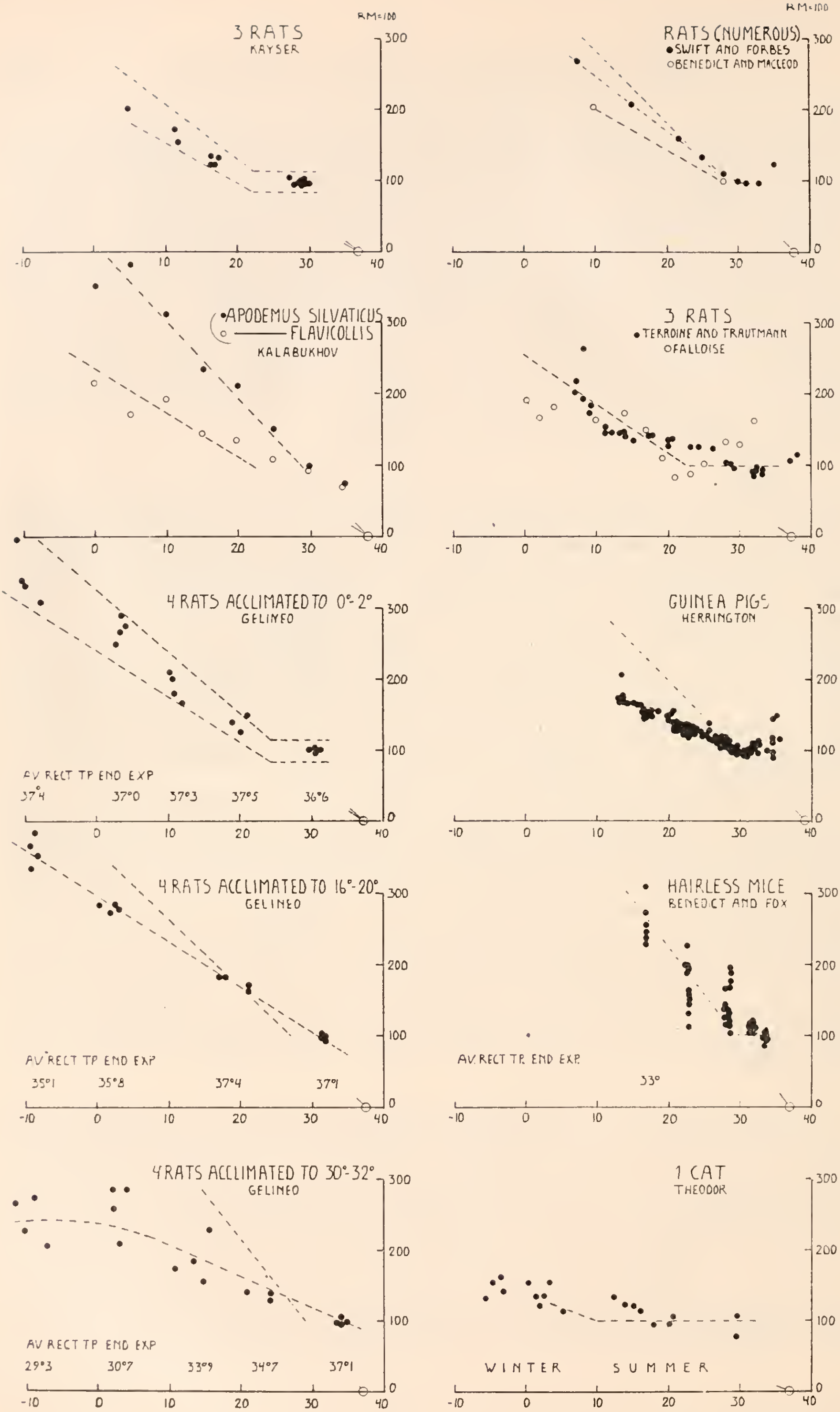
tremes Platypus and Echidna (Martin, 1902) and the Xenarthra, among which are the sloths and armadillos (Wislocki and Enders, 1935). Practically all others regulate to within ± 1°. However, when exposed to decreasing external temperature the central or main body temperature will finally fall, resulting in a relative lowering of the metabolism which drops from the line of proportionality. This is well illustrated in the three series of rat experiments of Gelineo replotted in Figure 8, and occurs in our jungle rat and night hawks (Fig. 6), and in Kendeigh's sparrows (Fig. 9), but is not apparent in Martin's Echidna (Fig. 7). Any change of insulation involves establishment of new temperature gradients and hence some change in the stored heat of the body. In large and well insulated animals, *e.g.,* man in heavy clothing, this may introduce considerable time lag before the new equilibrium is reached. All our animals were relatively small and the arctic ones, especially, were given considerable time for equilibrium.

2. *Insulation.* Insulation here means the sum total of all factors, such as fur, skin and tissue cooling, and posture, that impede the loss of heat from the animal. In order to obtain proportionality between heat production and temperature gradient below the critical temperature, the insulation must remain constant at its maximum value. This, however, is only approximate. During an experiment the animals make various changes in posture. They have periods of activity which not only influence the energy output, but also the overall insulation. In the numerous experiments where each curve is based on many animals, considerable spread of data can be expected because different individuals may have different critical temperatures. Furthermore, if the experiments have been conducted over a long period of time involving seasonal changes in the animals' insulation (moulting), we must expect only poor correlation (Theodor's cat, Fig. 8).

In all animals the insulation cover over different parts of the body varies within wide limits. Provided, however, that each of these parts at the critical temperature has developed and maintains its individual maximum insulation, it will essentially, from then on, lose heat in proportion to the gradient according to Fourier's law (McAdams, 1942), and hence Newton's law of cooling would still apply for the whole animal. As we shall see, Newton's law of cooling does hold pretty well for the whole animal below the critical temperature, and apparently each external part of the body is able to maintain a maximal insulation and a certain minimum temperature until the environmental temperature drops too low for maintenance of the body temperature. This is also indicated by the observations on the gull's feet mentioned in the previous paper.

3. *Heat Production.* The extra heat production required to maintain the body temperature below the critical temperature is mainly derived from muscular metabolism, either as shivering, gross activity, or both. It is essentially discontinuous, occurring in bursts, and as the animals may also have other reasons to move about during the experiments we must accordingly expect a considerable spread of data. It should be emphasized that our animals were confined in relatively small cages so

FIGURE 7. Metabolic heat regulation in mammals recalculated from literature. There is a fair overall adherence to Newton's law of cooling below the critical temperature. The echidna shows fair proportionality to gradient in spite of falling body temperature.


3 RATS
KAYSER
RM=100
RATS (NUMEROUS)
● SWIFT AND FORBES
○ BENEDICT AND MACLEOD
RM=100
● APODEMUS SILVATICUS
○ —— FLAVICOLLIS
KALABUKHOV
3 RATS
● TERROINE AND TRAUTMANN
○ FALLOISE
4 RATS ACCLIMATED TO 0°-2°
GELINEO
AV RECT TP END EXP
37°4
37°0
37°3
37°5
36°6
GUINEA PIGS
HERRINGTON
4 RATS ACCLIMATED TO 16°-20°
GELINEO
AV RECT TP END EXP
35°1
35°8
37°4
37°1
HAIRLESS MICE
BENEDICT AND FOX
AV. RECT TP. END EXP.
33°
4 RATS ACCLIMATED TO 30°-32°
GELINEO
AV RECT TP END EXP
29°3
30°7
33°9
34°7
37°1
1 CAT
THEODOR
WINTER
SUMMER
300
200
100
0
-10
0
10
20
30
40

that they could not perform any normal exercise in order to keep warm, like running around. This undoubtedly curbs their heat production and gives a false impression of their full natural capacity for metabolic heat regulation.

Experimental Results

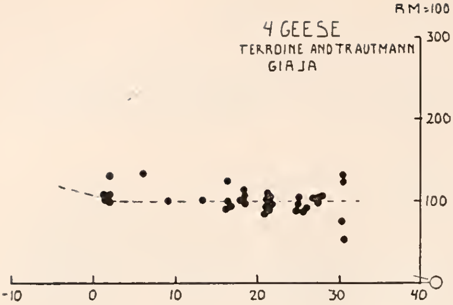
All of our own data, and all data from the literature that are sufficiently detailed, have been computed relative to a basal metabolic level in the thermo-neutral zone which we call 100, and are presented in Figures 4 through 9. Wherever possible, lines have been drawn according to the ideas put forth in Figure 3, so as to best fit the experimental data. It is evident that in the great majority of cases, going from arctic through temperate to tropical mammals and birds, the data fit the theory quite well. For a number of animals, especially in the arctic (fox, eskimo dog, polar bear, ground squirrel, arctic gull, snow bunting) and temperate regions (dog, rabbit, guinea pig, and goose), we see that there is a well defined zone of thermoneutrality extending in some of them down to − 30° C. This means that the insulation alone has compensated for the increasing gradient by a steady increase. From the observation that arctic dogs, foxes, and gulls sleep on the snow at − 40° C. to − 50° C., it is likely that their zone of thermoneutrality extends at least that far, which means that their changing heat dissipation alone can balance the heat loss from + 30° C. to − 40° C. If the foxes' body temperature is 37° C. this is equivalent to an 11-fold change in temperature gradient, and hence they can produce an 11-fold change in heat dissipation in spite of being confined in a small cage. Conversely of course, it follows that at − 40° C. they can increase their heat loss by at least eleven times the basal heat production in order to get rid of heat produced while running. While running freely they can probably dissipate heat considerably better, which may be very important at less severe temperatures. In other words, from their extended zone of thermoneutrality it directly follows that they are equally amazing in ability to dissipiate heat. A major factor in this ability must be vasodilatation and rise of skin temperature. An observation on eskimo dogs brought this out clearly. At − 3° C. air temperature the dogs could maintain a fur surface temperature, for some time, as high as + 26° C. (Table I). An eskimo dog that was brought to the States

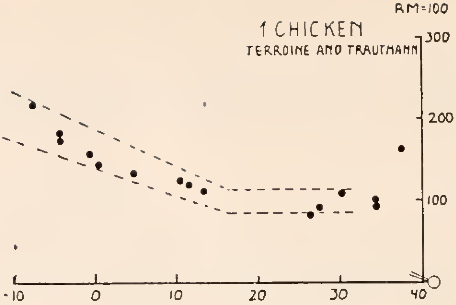
Table I

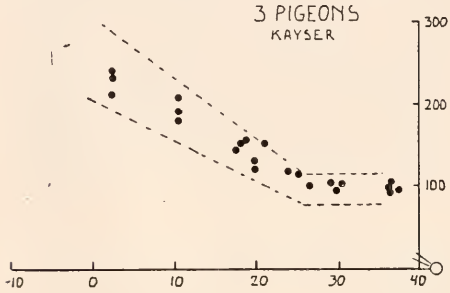
Simultaneous temperature measurements in adult eskimo dogs
(All temperatures Centigrade)

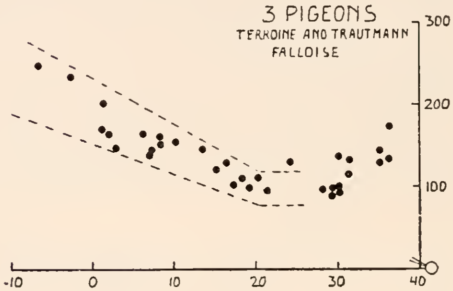
Dog	A	B	C	D	E
Rectal	38.3	38.3	38.2	38.4	40
Subcutaneous	37.2	37.1	37.6	37.4	38.2
Surface of skin	33	36	36.7	35.4	30.0
Outer limit of fur	10	6 to 13	26	23	−10
Air	−3	−3	−3	−3	−19

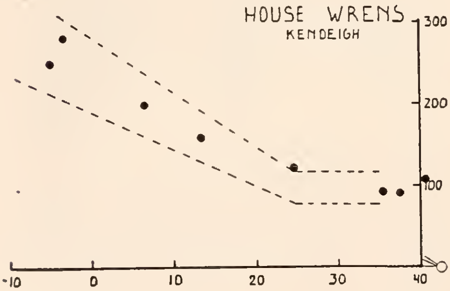
Figure 8. Metabolic heat regulation in mammals recalculated from literature. The metabolic rate drops off with falling body temperature in Gelineo's acclimation experiments. In the large rat series by Benedict and MacLeod and the guinea pig series by Herrington there is a poor correlation to the gradient.

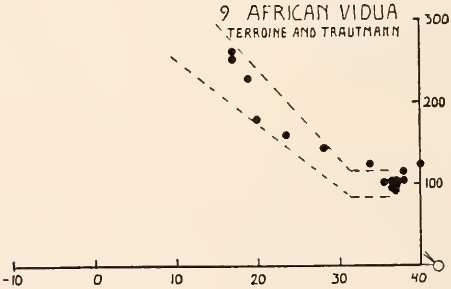

4 GEESE
TERROINE AND TRAUTMANN
GIAJA
RM=100
300
200
100
-10
0
10
20
30
40

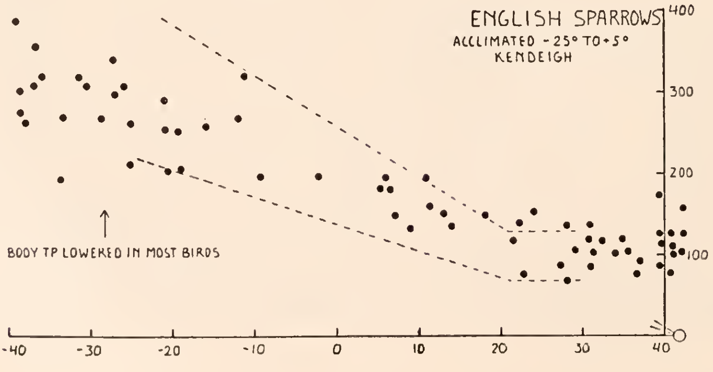

1 CHICKEN
TERROINE AND TRAUTMANN
RM=100
300
200
100
-10
0
10
20
30
40


3 PIGEONS
KAYSER
300
200
100
-10
0
10
20
30
40


3 PIGEONS
TERROINE AND TRAUTMANN
FALLOISE
300
200
100
-10
0
10
20
30
40


HOUSE WRENS
KENDEIGH
300
200
100
-10
0
10
20
30
40


9 AFRICAN VIDUA
TERROINE AND TRAUTMANN
300
200
100
-10
0
10
20
30
40


ENGLISH SPARROWS
ACCLIMATED -25° TO +5°
KENDEIGH
BODY TP LOWERED IN MOST BIRDS
400
300
200
100
-40
-30
-20
-10
0
10
20
30
40

in the heat of the summer got rid of his heat by stretching out on his back, exposing his thinly haired abdomen, and panting vigorously. The observed magnitude of change of heat dissipation in the fox matches the observation by Brody and Cunningham (1936) that the maximum steady state of muscular activity in man and horse is of the order of 10–15 or even 20 times the resting metabolism. It was pointed out in the previous paper that the poorly insulated legs of the arctic mammals and birds are probably a major factor in the heat regulation of these animals.

In most of the tropical animals the zone of thermoneutrality is so limited and transitory that the two parallel lines drawn in the diagrams represent more of an abstraction than a reality. In some cases the transition between the zone of thermoneutrality and metabolic rise is definitely a curve (see Goto's rats, Herrington's mice, Fig. 7). Such a thing would happen also in our insulated model if the metabolic rise started before the maximum of the insulation had been reached. This may be a reason for the low slope in the rat experiments of Benedict and MacLeod and of Swift and Forbes (Fig. 8), and in the guinea pig experiments of Herrington (Fig. 8). Another reason may be a possible drop in body temperature at the lowest temperatures. Such a drop of the curves due to fall in body temperature is illustrated in Gelineo's rat experiments (Fig. 8), in Kendeigh's sparrows (Fig. 9), and in our jungle rat and night hawks (Fig. 6).[8] In the scant data on the platypus and the three Australian marsupials (Martin, Fig. 7), there is an upward trend in the curves. Because of the inherent spread in all similar experiments it seems doubtful that this trend is significant, but it could happen if the insulation deteriorated markedly with activity as may occur with human clothing.

As has been described, there is a considerable spread in most of the data and also a tendency to proportionately greater spread during the period of metabolic heat regulation, when bursts of activity rather than an even flow supply the heat which maintains the body temperature. The maximum amount of heat produced above the base value is undoubtedly influenced by time, and in long-time experiments the peak metabolism is seldom over 400 (Echidna) and usually not more than 300 per cent. In most cases, however, the high peak values (*metabolisme du sommet*) found after acute chilling in ice water (Giaja, 1925) cannot be produced in prolonged experiments. Animals at liberty to move about freely can, however, undoubtedly maintain a higher metabolic rate than under the cramped conditions of an experiment.

With the facilities available at the Arctic Research Laboratory at Point Barrow we were not able to reach the critical temperature for the foxes or dogs. However, with excellent cooperation from the Navy, two foxes and several snow buntings were flown to the Naval Research Laboratory, Washington, D. C., and a series of observations was made at temperatures down to − 80° C. (Table II). From the table it is apparent that − 70° C. induced shivering and is therefore below the critical temperature of the fox. He suffered no loss of body temperature after one hour's

---

[8] The drop in body temperature and poor metabolic response to the lowered outside temperature observed in this goatsucker may well be due to a hibernation reaction, as the related poor-will (*Phalaenoptilus nuttallii*) is known to hibernate (Amadon, 1949). This information was kindly furnished by Dr. R. C. Murphy of the American Museum of Natural History.

FIGURE 9. Metabolic heat regulation in birds recalculated from literature. Many of Kendeigh's sparrows drop off from the line of proportionality because of drop in body temperature, and many died after the severest cold exposure. *Vidua paradisea* (Terroine and Trautmann, 1927) is a bird native to equatorial West Africa (Delacour and Edmond-Blanc, 1933, 1934).

TABLE 11

Rectal temperature in white fox subjected to low temperature
(All temperatures Centigrade)

Air temp.	Duration in minutes	Rect. temp. before exp.	Rect. temp. end exp.	Remarks
+22		37°7		
−20		37°7		
−50	120	37–39°	37°6	Lying down asleep.
−60	105	37–39°	39°	Lying down asleep.
−70	90	39°	37°3	Licked feet ½ hour, curled up asleep, shivering after 1 hour.
−80	60	37–39°	38°	Licked himself, asleep, shivering after 5 minutes. Legs stiff, active.

stay at − 80° C., and his metabolic heat regulation can probably balance considerably lower temperatures, but he may suffer local frost injury long before exhaustion of his metabolic resources. From observations of sleeping foxes and eskimo dogs it is believed that their critical temperature is somewhere between − 45° C. and − 50° C., and it is most likely the same in all the other larger mammals of the same climate. Snow buntings, which normally migrate south in the winter, could not take − 50° C. and their body temperature dropped seriously within an hour.

The data on all our arctic and tropical animals are collected in charts, Figures 10 and 11, where the full drawn lines represent observations and the dotted lines represent extrapolation of the metabolism below a known critical temperature.

The animals most sensitive to low temperature have the steepest curves, with critical temperatures near the body temperature. The animals hardiest toward cold have the least slope, with critical temperatures farthest from the body temperature. We have noted that the cold hardiest animals, the fox and eskimo dog, can be expected to tolerate the coldest temperatures on earth merely by increasing their me-

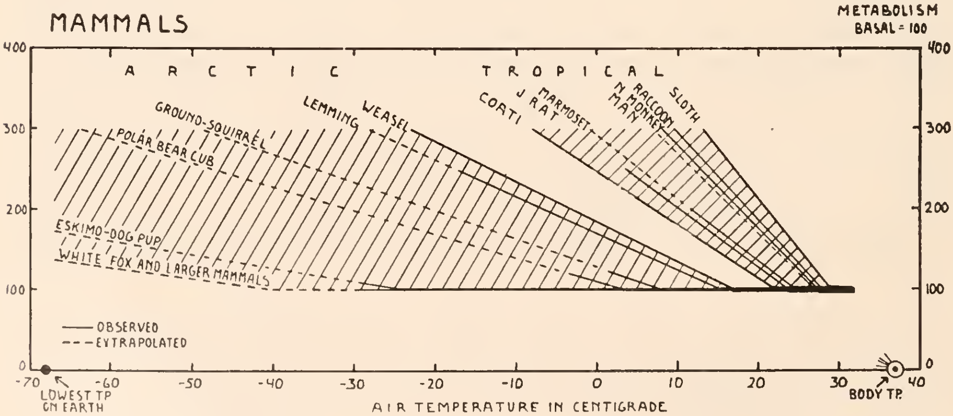


FIGURE 10. Heat regulation and temperature sensitivity in arctic and tropical mammals based on plots in Figures 4, 5, and 6. The fox needs only slight increase in metabolic rate to stand the coldest temperature on earth. The critical gradients (tp sensitivity) and the slope of the curves depend on the product of the basal metabolic rate and the overall body insulation.

tabolism 30–40 per cent, whereas the same increase in metabolism in a tropical animal will only help him by a few degrees.

The critical temperature in naked man is known to be around 27° C. (DuBois, 1936) to 29° C. (Winslow and Herrington, 1949), which places him among the more temperature sensitive of the tropical mammals. We have no data as to the critical temperature of Eskimos. They wear just as warm clothing as white men in the same environment, often warmer, and it is likely that the Eskimo is as sensitive to temperature as other people that wear clothing. Man is indeed a tropical animal carrying his tropical environment with him.

By using different amounts of clothing, tropical man can span the whole range from the tropics to the arctic and it could be assumed that his energy requirements below the critical temperature for each clothing assembly would essentially follow Newton's law of cooling. It seems a challenge that we have been unable to unearth in the literature experiments in man, naked or clothed, with enough data to demonstrate adequately the thermoneutral zone and the slope of energy expenditure beyond it. In Figure 10 man's line below the critical temperature is hence dotted. It is known however, from studies at the Climatic Research Laboratory, Lawrence, Massachusetts, that the insulation value of clothing may drop considerably (50 per cent) during exercise (Belding, 1949, and personal communication), which would move the curves upward. Man cannot usually spend a night at − 40° C. in one-layer arctic fur clothing or naked in a one-layer arctic fur sleeping bag without getting cold and shivering. He needs more insulation than the animals whose fur he is using. The reasons may be several. In his outstretched sleeping position he exposes much more surface than an arctic mammal sleeping curled up. The arctic mammals may tolerate a low leg temperature, like the arctic gull, and thereby greatly conserve heat. Fur clothing and fur sleeping bags are usually not made from the heaviest winter fur, but rather from lighter autumn fur with less insulating value.

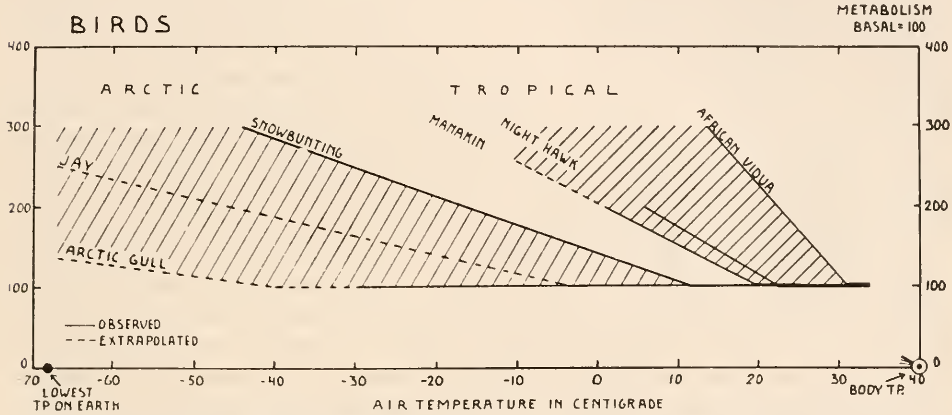


FIGURE 11. Heat regulation and temperature sensitivity in arctic and tropical birds, based on plots in Figures 5, 6, and 9.

It is readily seen by comparing Figures 10 and 11 of this paper with Figure 3 in the preceding paper that the correlation between the measured insulation of a piece of fur of the animal and its temperature sensitivity does not hold in detail.

Thus the smaller arctic animals are considerably less temperature sensitive than the equally well or better insulated tropical mammals.

From Figure 3 it is clear that the temperature sensitivity, *i.e.*, critical gradient, depends upon the product of the overall insulation and the basal metabolic level. The overall insulation is only roughly reflected by measurements of the fur insulation on a patch taken from the body side or back, but even with this limitation we shall see that most of the apparent discrepancies can be explained by considering the metabolic level.

SUMMARY

A series of arctic and tropical mammals and birds at Point Barrow, Alaska (lat. 71° N.) and in Panama (lat. 9° N.) was subjected to various air temperatures in a respiration chamber where the heat production was determined by oxygen consumption or carbon dioxide production. The larger arctic mammals and birds showed no increase in metabolism at − 30° C. and from observations on sleeping animals it is probable that their zone of thermoneutrality extends to − 40° C. or − 50° C. The smaller arctic species show a high critical temperature and the tropical species even higher. Metabolic heat production increases rapidly with lowering of the temperature in a tropical mammal or bird, and slowly in an arctic animal. It can be shown theoretically that in a thermoregulated system with a fixed basal energy level and variable insulation the critical gradient is proportional to the maximal insulation and the basal energy level.

In a large series of experiments including our tropical and arctic animals, and all animals affording enough data in the literature, it is shown that the heat loss below the critical temperature is essentially proportional to the body-to-air gradient. This means that the overall insulation evidently reaches a maximum at the critical temperature and from then on the heat loss follows essentially Newton's law of cooling. It follows from this that an arctic mammal with a critical gradient of 70° C., by doubling its metabolism, theoretically would double the gradient. Only 40 per cent increase of its metabolism (or insulation) would suffice to take it down to − 70° C. which is near the lowest recorded temperature on earth.

The very broad zone of thermoneutrality in the larger arctic species, from + 30° C. to − 40° C., shows their ability to balance an 11-fold increase in gradient and hence the animal can change its heat dissipation by a factor of 11 even when lying down. It is believed that vasomotor control of the poorly insulated legs must play an important role in the general thermoregulation of these animals.

In the tropical mammals and birds the critical gradient is low, often only 10° C., which makes them sensitive to even small temperature changes. A 10° lowering of the air temperature from the critical temperature doubles the gradient for the tropical mammal; a 9° increase decreases the gradient 10 times, and in order to maintain the body temperature tropical animals must be able to adjust insulation and metabolism in the same proportion as the gradient. They are thus extremely sensitive to temperature changes.

The whole range of heat regulation from tropical to arctic mammals and birds is represented on two charts, Figures 10 and 11.

ACKNOWLEDGMENT

We are greatly indebted to the officers, civilians and natives of the Barrow camp for their many services. We wish to acknowledge likewise the excellent cooperation and facilities afforded us by Captain H. W. Taylor, USN, Commanding Officer of the U. S. Naval Air Station, Coco Solo, Canal Zone. We are also greatly indebted to Mr. James Zetek, U. S. Department of Agriculture, Balboa, Canal Zone, for helping us in every way at the Barro Colorado Laboratory. We are grateful to Mr. Walter Flagg and to Mr. R. H. Stapleton for much capable assistance and to Dr. Harald Erikson for permission to use some of his ground squirrel data. While preparing the data we have had the privilege of stimulating discussions with Dr. Alfred C. Redfield, Dr. Cecil K. Drinker and Dr. Alan C. Burton.

Addendum

After the manuscript and illustrations were all prepared, Mr. Charles M. Bogert at the American Museum of Natural History kindly made us aware of an important paper appearing in the latest available issue of *Bullétin de l'Académie des Sciences de l'U.R.S.S.*, Volume 2, 1947, by R. P. Olnianskaya and A. D. Slonim. They subjected several zoo animals at Leningrad to temperatures from + 20° C. to − 20° C., measuring metabolism and the rectal temperatures: *Vulpes vulpes, V. lagopus, V. melanotus, Lepus europaeus, L. timidus,* and *Nyctoreutes procyonoides.* The polar fox showed only insignificant rise in metabolism from + 20° C. to − 20° C., the red fox a 50 per cent rise, and the other animals more. There is a fair correlation between gradient and metabolic rate in their curves. Absolute metabolic rates are not given. The authors conclude that the polar species have a more constant body temperature at low environmental temperatures than the southern species, and that the metabolism rises only little in the polar species as compared with the southern species. The maintenance of constant body temperature in the arctic species is considered to be due to an extraordinary vascular control. Insulation is not mentioned.

LITERATURE CITED

Amadon, D., 1949. Comments on recent literature. *The Wilson Bulletin,* **61**: 237.

Belding, H. B., 1949. Protection against dry cold. Quartermaster Climatic Research Lab., Rept. No. **155**. Lawrence, Mass.

Benedict, F .G. and E. L. Fox, 1933. Der Energieumsatz normaler und haarloser Mäuse bei verschiedener Umgebungstemperatur. *Pflügers Arch. Gesamte Physiol.,* **231**: 455.

Benedict, F. G. and Grace MacLeod, 1929. The heat production of the albino rat. I. and II. *Jour. Nutrition,* **1**: 343 and 367.

Brody, S., 1945. Bioenergetics and growth. New York.

Brody, S. and R. Cunningham, 1936. Growth and development, with special reference to domestic animals: XL. Comparison between efficiency of horse, man, and motor, with special reference to size and monetary economy. *Res. Bull. Agr. Exp. Sta.,* Univ. of Missouri, **244**: 5.

Burton, A. C., 1934. The application of the theory of heat flow to the study of energy metabolism. *Jour. Nutrition,* **7**: 497.

Delacour, J. and F. Edmond-Blanc, 1933. Monographie des veuves. *Ois. Rev. Française Ornith.,* **3**: 3 and 4.

Delacour, J. and F. Edmond-Blanc, 1934. Monographie des veuves. *Ois. Rev. Française Ornith.,* **4**: 1.

Dhar, N. R., 1926. Influence of temperature on metabolism and the problem of acclimatization. *Allahabad Univ. Studies,* **2**: 313.

Du Bois, E. F., 1936. Basal metabolism in health and disease. Philadelphia.

Erikson, H., 1950. Unpublished data.

Falloise, A., 1900. Influence de la température extérieure sur les échanges respiratoires chez les animaux à sang chaud et chez l'homme. *Arch. Biol.,* **17**: 761.

Gelineo, M. S., 1934. Influence du milieu thermique d'adaptation sur la thermogénèse des homéothermes. *Ann. Physiol. Physicochim. Biol.,* **10**: 1083.

Giaja, J., 1925. Le métabolisme de sommet et le quotient métabolique. *Ann. Physiol. Physicochim. Biol.* **1**: 596.

Giaja, A., 1931. Contribution à l'étude de la thermorégulation des oiseaux. *Ann. Physiol. Physicochim. Biol.*, **7**: 13.

Goto, K., 1923. Beitrag zur Kenntnis der chemischen Wärmeregulation der Säugetiere. *Biochem. Zeitschr.*, **135**: 107.

Herrington, L. P., 1940. The heat regulation of small laboratory animals at various environmental temperatures. *Amer. Jour. Physiol.*, **129**: 123.

Hock, R. J., 1949. Temperature and bat metabolism. Unpublished doctoral thesis, Cornell Univ.

Hoesslin, H. v., 1888. Über die Ursache der scheinbaren Abhängigkeit des Umsatzes von der Grösse der Körperoberfläche. *Arch. Anat. Physiol., Physiol. Abth.*, p. 323.

Kalabukhov, N. I., 1940. Influence of temperature upon oxygen consumption by wood-mouse (*Apodemus silvaticus* L.) and yellow-necked mouse (*A. flavicollis* Melch.). *Compt. Rendus Acad. Sci. URSS,* **26**: 89.

Kayser, C., 1930. Contribution à l'étude de la régulation thermique. *Ann. Physiol. Physicochim. Biol.*, **6**: 721.

Kayser, C., 1937. Variations du quotient respiratoire en fonction de la température du milieu chez le rat, le pigeon et le cobaye. *Compt. Rendus Soc. Biol.*, Paris, **126**: 1219.

Kendeigh, S. C., 1939. The relation of metabolism to the development of temperature regulation in birds. *Jour. Exp. Zool.*, **82**: 419.

Kendeigh, S. C., 1944. Effect of air temperature on the rate of energy metabolism in the English sparrow. *Jour. Exp. Zool.*, **96**: 1.

Kleiber, M. and J. E. Dougherty, 1934. The influence of environmental temperature on the utilization of food energy in baby chicks. *Jour. General Physiol.*, **17**: 701.

Lapicque, L., 1921. Échanges nutritifs des animaux en fonction du poids corporel. *Compt. Rendus Acad. Sci.*, Paris, **172**: 1526.

Lefèvre, J., 1911. Chaleur animale et bioenergétique. Paris.

McAdams, W. H., 1942. Heat transmission. New York.

Martin, C. J., 1902. Thermal adjustment and respiratory exchange in monotremes and marsupials. *Phil. Trans. Roy. Soc. London*, B, **195**: 1.

Mitchell, A. C., 1901. On the convection of heat by air currents. *Trans. Roy. Soc. Edinburgh,* **40**: 39.

Olnianskaya, R. P. and A. D. Slonim, 1947. On the adaptability of animal organisms to very low temperatures of the environment. *Bull. Acad. Sci. URSS*, ser. Biol., **2**: 245.

Rubner, M., 1902. Die Gesetze des Energieverbrauches bei der Ernährung. Berlin-Wien. (Quoted from Otto Kestner and Rahel Plaut, Physiologie des Stoffwechsels, *in* H. Winterstein, *Handbuch der Vergleichenden Physiologie*, **2**: 901, 1924. Jena.)

Scholander, P. F., 1947. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. *Jour. Biol. Chem.*, **167**: 235.

Swift, R. W. and R. M. Forbes, 1939. The heat production of the fasting rat in relation to the environmental temperature. *Jour. Nutrition*, **18**: 307.

Terroine, E. F. and S. Trautmann, 1927. Influence de la température extérieure sur la production calorique des homéothermes et loi des surfaces. *Ann. Physiol. Physicochim. Biol.,* **3**: 422.

Terroine, E. F. and E. Zunz, 1925. Le métabolisme de base. Les Presses Universitaires de France, Paris.

Theodor, C., 1878. Über den Einfluss der Temperatur der umgebenden Luft auf die Kohlensäureausscheidung und die Sauerstoffaufnahme bei einer Katze. *Zeitschr. Biol.*, **14**: 51.

Wetmore, A., 1921. A study of the body temperature of birds. *Smithsonian Misc. Coll.*, **72** (12): 1.

Winslow, C. E. A., A. P. Gagge, L. Greenburg, I. M. Moriyama and E. J. Rodee, 1935. The calibration of the thermo-integrator. *Amer. Jour. Hygiene*, **22**: 137.

Winslow, C. E. A. and L. P. Herrington, 1949. Temperature and human life. Princeton.

Wislocki, G. B., 1933. Location of the testes and body temperature in mammals. *Quart. Rev. Biol.,* **8**: 385.

Wislocki, G. B. and R. K. Enders, 1935. Body temperatures of sloths, anteaters, and armadillos. *Jour. Mammal.*, **16**: 328.

ADAPTATION TO COLD IN ARCTIC AND TROPICAL MAMMALS AND BIRDS IN RELATION TO BODY TEMPERATURE, INSULATION, AND BASAL METABOLIC RATE *

P. F. SCHOLANDER,[1] RAYMOND HOCK,[2] VLADIMIR WALTERS,[3] AND LAURENCE IRVING [4]

In two previous papers [5] we showed how climatic adaptation of arctic and tropical mammals and birds was clearly manifested by their widely different critical gradients. The critical gradient was defined as the largest temperature difference between the body interior and the air that an animal can stand without losing body temperature, when the metabolism is basal.

It was pointed out that the critical gradient in an insulated thermoregulated system depends upon the product of the over-all insulation and the basal metabolic rate. This leaves the animals with three possible main avenues for adaptation to cold, namely (1) the body-to-air gradient, (2) the insulation, and (3) the basal metabolic rate, and it is our task in the present paper to estimate quantitatively the importance of each of these factors for climatic adaptation. For this purpose we must first know more precisely what thermal stresses animals are faced with in their arctic and tropical climate.

The Thermal Environment

The temperature conditions at Point Barrow and at Barro Colorado are about as different as could be found. Records shown in Table I indicate that on the arctic coast annual extremes may differ by 65°. Inland or in exceptional years on the coast the difference may be 80°–90°.

At Barro Colorado the temperature in a shaded location near the ground is extremely constant and seldom varies from 28° C. Tropical microclimates, on the other hand, vary considerably, as is shown by observed diurnal variations with altitude in the forest at Barro Colorado. In February the diurnal variation at ground level was 6.5°, at a 55 foot height 9.5°, and at an 86 foot altitude 16.5° (Allee, 1926). The mean maximum-minimum range at ground level is 2.9° and in the canopy 11.8° or four times greater.

On first consideration we would expect an arctic mammal to be cold adapted and a tropical mammal to be heat adapted. It is quite obvious that the arctic mammals are adapted to withstand cold or they would freeze to death. If we consider, how-

---

* The experimental work was supported through contract with the Office of Naval Research. The preparation of the material for publication was assisted by the Arctic Health Research Center, U. S. Public Health Service.

[1] Now at Department of Biological Chemistry, Harvard Medical School.

[2] Now at Arctic Health Research Center, Anchorage, Alaska.

[3] Now at Department of Biology, New York University.

[4] Now at Arctic Health Research Center, Anchorage, Alaska.

[5] Many problems touched upon in this and the foregoing papers have been discussed in the stimulating paper "The application of the theory of heat flow to the study of energy metabolism" by Burton (1934).

ever, that fur always conserves heat and that most tropical mammals are fur covered, then we realize that conservation of heat, *i.e.*, adaptation to cold, is also a conspicuous feature in tropical mammals. We may say then that an arctic mammal is adapted to cold and a tropical mammal to less cold. That this is true is evident if we consider the critical gradients. Several of the tropical mammals examined have a critical temperature between 25° C. and 27° C. which is only 10° below their body temperature. If the air temperature drops only a few degrees lower they begin to shiver from cold, and if it were to rise only 9° above their critical temperature they would have to increase their heat dissipation ten times to keep in heat balance. Such changes in environmental temperature do, however, take place between day and night in the tropics (Allee, 1926). In contrast to this an arctic mammal at its critical temperature of − 40° C. to − 45° C. can adjust to 0° C. merely by halving the insulation. Such large changes in the environmental temperature are seasonal rather than diurnal, however, and are compensated for by seasonal changes in insulation (shedding).

It seems then that the problem for tropical mammals is neither overheating nor cooling but, actually, both. Their low critical gradient, whether due to low insulation or low metabolism, gives them a very small range of temperature tolerance in both directions. The heat of the day, rain and storm, and the cool of the night will frequently exceed their narrow zone of thermoneutrality and in short succession they may be subjected to both cold and heat. The same is often experienced by man in the tropics. The days may be extremely hot even with a minimum amount of clothing, and a night without extra clothes can be very chilling. What then should a tropical mammal adapt for, overheating in day or cooling at night? The fact that most of them are well furred shows that they do adapt to cold. By what means and to what extent they can protect themselves against heat, we have not studied quantitatively. Some of them do it by sweating and panting, many avoid the sun by seeking shade, and many are inactive during the heat of the day; some resort to the water for cooling. It would seem that gains in latitude of heat regulation would be an advantageous adaptation to a tropical climate.

TABLE I

Temperature reported at Barrow, Alaska (lat. 71°) in 1948, converted to centigrade degrees (from climatological data, Alaska, 1948)

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Average	−28	−24	−27	−17	−10	− 1	5	2	− 4	−12	−21	−29
Minimum	−38	−42	−43	−32	−26	−10	− 2	− 5	−15	−26	−40	−43
Maximum	− 5	− 4	−13	1	0	4	22	14	5	− 2	− 2	− 8

We pointed out above how cold sensitivity would depend upon three main factors: (1) the body-to-air gradient, (2) the insulation, and (3) the basal metabolic rate.

BODY TEMPERATURE AND CLIMATIC ADAPTATION

We shall first consider the body-to-air gradient in relation to climatic adaptation. As the heat loss is proportional to the gradient, other factors being equal, arctic mammals would gain in heat conservation by having a lower body temperature than other mammals, and tropical mammals would gain in latitude of heat regulation if they were warmer than usual.

We saw in the preceding papers that all arctic mammals and birds so far tested have an internal body temperature like the temperate forms, and actually they would gain very little in heat preservation from a low body temperature unless it were considerably lower than normal. A body temperature of 30° C. instead of 37° C. would, at − 40° C. air temperature, save only 10 per cent of the rate of heat loss. The large lowering of the body temperature during hibernation combined with an insulated nest does, however, reduce the gradient and the heat loss considerably, but it occurs only in dormant animals. The smaller arctic mammals and man could not maintain inactive resting periods without lowering the gradient, however, and they can only do this if they raise the external temperature by providing themselves with added insulation in the form of nests and clothing. It is interesting to note that "behavioral thermoregulation" has also been found to be characteristic for all reptiles thus far studied (Bogert, 1949).

There are no tropical mammals or birds known to have body temperatures higher than usual (Wetmore, 1921; Wislocki, 1933), and consequently none of them have a heat adapted body temperature. Quite a few normally have a low body temperature, however, *e.g.*, sloths, armadillos, anteaters (Wislocki and Enders, 1935), and monotremes (Martin, 1902). Lowered body temperature *per se* makes them a little more cold hardy, but at the same time more sensitive to changes in the air temperature because of the lowered critical gradient. They gain some latitude, however, by the fact that they are all poor temperature regulators. This is a condition well known in bats and other hibernators from all climates and represents traces of a thermal survival latitude characteristic of poikilotherms. It has nothing to do with an adaptive change of body temperature to a new and constant level such as we would expect in a truly homoiothermic animal. We may state then that there are no signs so far that body temperature of mammals and birds is adaptive to the different climates on earth. A logical corollary of this is that it cannot have been adaptive to the over-all climatic conditions on earth either. It seems then that the narrow band of body temperature on which both birds and mammals operate is a fundamental, nonadaptive constant in their biochemical setup. It can be kept constant only within certain climatic limits which are determined by the latitude of the physical and chemical heat regulation of the animal. Whereas mammals and birds undoubtedly could adapt to colder climates than we have today by increasing their insulation, they are very near their upper limit in a warm tropical climate. Many parts of the tropics are so hot and humid that a few degrees' rise in the temperature would mean death for mammals and birds because they cannot adapt to it by raising their body temperature.

In contrast to the inadaptability of the internal body temperature, peripheral parts may show a great deal of temperature adaptation, such as the cold extremities of aquatic mammals and birds.

Insulation and Climatic Adaptation

Having eliminated the internal body temperature as an adaptive factor in arctic and tropical mammals, there remains the insulation and the basal metabolism to account for the striking differences which we have observed in their sensitivity to cold. It is, of course, common knowledge that the mammalian body insulation is highly adaptive to climate, phylogenetically as well as ontogenetically. We saw that the mammalian species of cold climates have, in general, much warmer fur than those of warm climates; that there are adaptive seasonal changes (moulting), and it is known that, for instance, dogs (v. Hoesslin, 1888) and rabbits (Mayer and Nichita, 1929) can be experimentally induced to increase their insulation if exposed long enough to cold. There is no reason to believe that the arctic climate is so cold that the larger mammals cannot produce adequate insulation against it but must resort to a high metabolism. They could certainly grow and wear much longer and warmer fur if they needed to, *i.e.,* a 500 kg. moose would not need to stop at the insulation of a 5 kg. fox.

In a previous paper we substantiated, by insulation measurements, the general rule that arctic mammals have warmer furs than do the tropical mammals. We found, however, some striking exceptions to this. For instance, the little arctic weasel, although much cold hardier, possesses only half the fur insulation of the tropical sloth. We pointed out that the explanation was to be found in the difference in the heat production of the two animals. The cold legs of arctic aquatic birds and mammals (and probably of the terrestrial forms as well) may be taken as another example of adaptive insulation.

Basal Metabolic Rate and Climatic Adaptation

As pointed out by Rubner (1883), Richet (1891), Voit (1901) and others, practically all mammals so far investigated show a nearly linear relation between the basal heat production and the body surface, usually estimated according to Meeh's formula as $K \times \text{Weight}^{2/3}$. They were found, in other words, to have approximately the same rate of heat dissipation per surface unit, or to follow the "surface law" of Rubner. The material was later amplified by Benedict (1938) in his famous mouse to elephant curve, and it was realized that the correlation came closer to $K \times \text{Weight}^{3/4}$ (Kleiber, 1932, 1947; Brody and Procter, 1932), which does not have the meaning of a surface area, although it is numerically almost the same except at the extremes of size. The fact that a near surface relation between body size and basal metabolic rate holds also for many groups of cold-blooded animals (Weymouth, Crismon, Hall, Belding and Field, 1944) is a clear indication that this relation is fundamental and is not primarily caused by heat loss (Terroine and Delpech, 1931; Krogh, 1941; Zeuthen, 1947).

From Benedict's mouse to elephant curve it is indicated that the basal metabolic rate is determined by body size, irrespective of climate. If this is so, however, and the body temperature is constant, then we may conclude that the only factor left to take care of climatic adaptation is insulation. Without further evidence we cannot preclude, however, that one might still find different adaptive metabolic levels in animals living under such extremes as the arctic and the tropics. A higher heat production, other factors being equal, would make an animal cold-hardier, and a lower heat production heat-hardier, and it is of interest therefore to know the metabolic rate of our animals in absolute units as compared with animals from less extreme climates.

In Figures 1 and 2 we have plotted our arctic and tropical mammals on the standard mouse to elephant curve, drawn according to the equation Cal./per day = 70 kg.$^{3/4}$. The metabolism has been calculated from the oxygen consumption (arctic) or the CO_2 production (tropical), assuming an RQ of 0.80, and a caloric equivalent of one liter of oxygen = 4.8 Calories. Generally when such determinations are made the animal is confined in a cage but can move about during the experiment if it so wishes. Even if active runs are weeded out, the strict prerequisites for a basal determination as in man rarely apply. However, we believe that our determinations fall in line with most other determinations of the basal energy level in wild animals, with the added advantage that we know definitely that each determination was made in the thermoneutral zone. Most of our determinations were started in the morning, the larger animals having fasted over night, the smaller ones for a couple of hours.

From Figure 2 we see that our adult arctic mammals, lemmings, ground squirrels, and foxes all line up closely with the standard curve, but the weasels do not. Since the larger arctic mammals (from a 5 kg. fox to a 500 kg. moose) have about the same

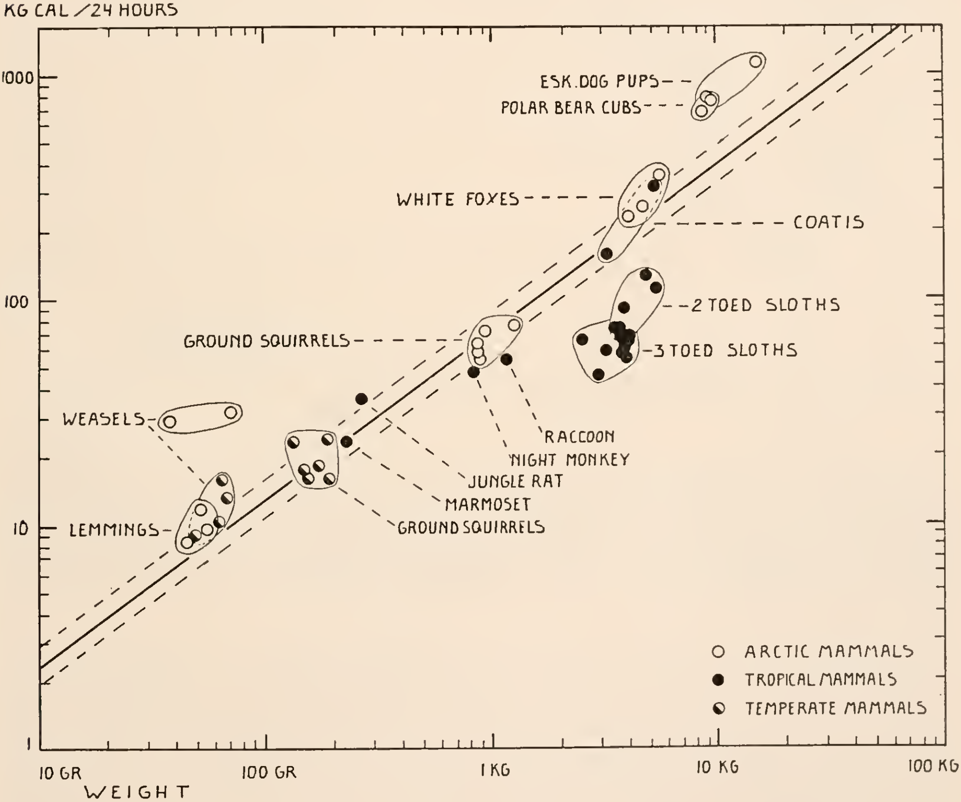


FIGURE 1. Basal metabolic rate in relation to body size. Full drawn line is the mouse to elephant curve drawn according to the equation Kg. Cal./day = 70 × kg.$^{3/4}$. Dotted parallel lines are 20 per cent deviations. Besides our own material the following has been used: ground squirrels (Erikson, 1950; and Gelineo, 1939), sloths (Ozorio de Almeida and Branca de Fialho, 1924; Irving, Scholander, and Grinnell, 1942), least weasels from Wisconsin (Morrison, unpublished).

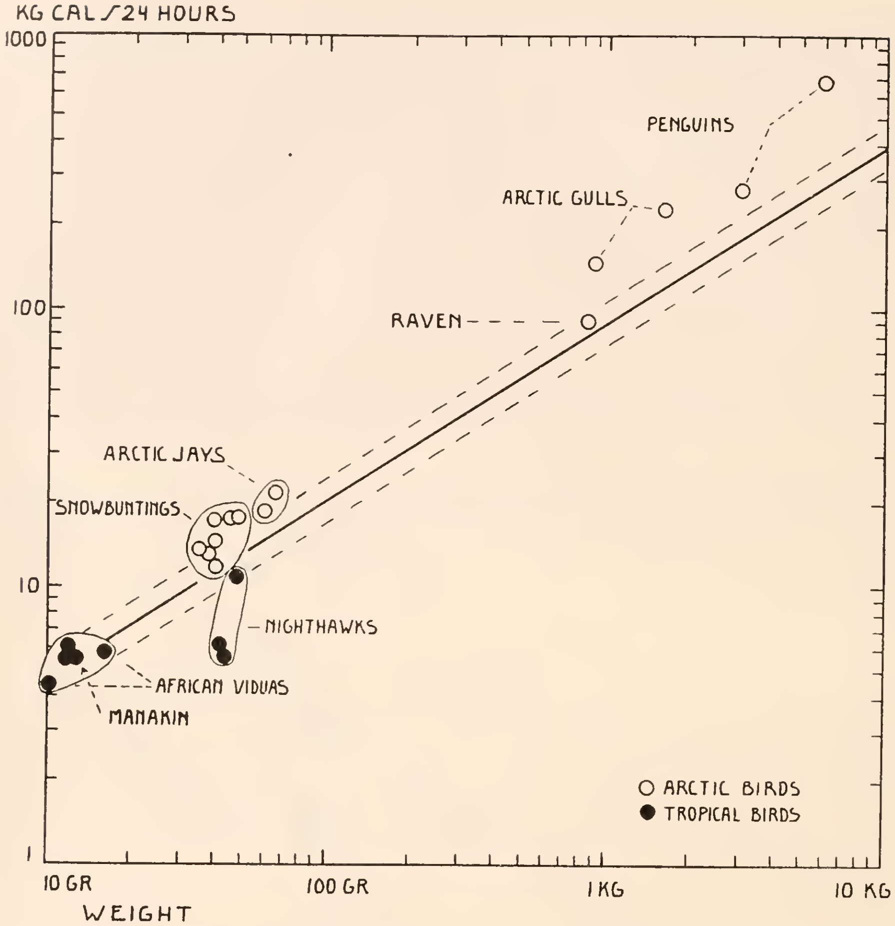


FIGURE 2. Basal metabolic rate in relation to body size. Full drawn line represents the curve given by Brody (1945) for birds according to the equation Kg.Cal./day = $89 \times \text{kg.}^{0.64}$. Dotted parallel lines are 20 per cent deviations. The African *Vidua paradisea* are taken from Terroine and Trautmann (1927), the antarctic penguins (*Pygoscelis papua* and *Eudyptes chrysotophus*) from Scholander (1940). The raven was kept most of the time as a pet in the laboratory building (− 5° C. to + 25° C.).

fur insulation per unit surface, and since they are subjected to the same climatic temperature gradients, it follows that their heat loss will be roughly proportional to their surface area. Since the fox falls on the mouse to elephant line it is therefore likely that all the larger arctic mammals do also. We see further that the tropical mammals, excepting the two species of sloths, likewise fall on the line. If we realize furthermore that the mouse to elephant curve already is based on animals ranging from relatively cold-hardy temperate rabbits to tropical elephants we may state as a tentative generalization that *the basal metabolic rate of terrestrial mammals from tropics to arctic is fundamentally determined by a size relation according to the* *formula* $Cal./day = 70\ kg.^{3/4}$*, and is phylogenetically nonadaptive to external temperature conditions. Equally nonadaptive is the body temperature, and the phylogenetic adaptation to cold therefore rests entirely upon the plasticity of the factors which determine the heat loss, mainly the fur insulation.* Small adaptive changes cannot, of course, be detected by such interspecific comparisons as these. There is also, however, intraspecific evidence from observations on man, which have failed to reveal any certain racial metabolic or body temperature adaptations to cold climates (DuBois, 1936). Man and animal alike do it all with insulation.

Our weasels were adults and had a metabolic rate two to three times higher than that expected from the standard curve. There are indications that our weasel figures are representative. The figures are averages of the lowest of several determinations in which the weasel was seen to be lying down curled up. Furthermore, during cold experiments they mobilized a heat production nearly four times larger than basal, which is as high as any other animal could maintain for long periods in a small cage. Finally, as we shall see, the high metabolism is a corollary to their relatively poor body insulation. Dr. Peter R. Morrison has kindly informed us that the average minimum value from runs on two individuals (*Mustela rixosa*) from Wisconsin showed only a slight elevation above the standard curve (Fig. 1). Hence we are forced to conclude that our arctic weasels had a greatly increased resting metabolic rate compared with the southern form. This may possibly mean a metabolic adjustment to cold. If the cold were the direct reason for the high resting rate it seems somewhat odd that the fur is so slight; presumably it could easily have been doubled!

We must still account for the fact that some tropical mammals have a very low metabolic rate, notably the sloth and possibly the armadillo. The sloth is clearly adapted to cold because of its warm fur, which acts compensatory to its low metabolism. Its low body temperature is likewise heat conserving. Low metabolism combined with low or labile body temperature is found also in non-tropical mammals, *e.g.,* marmot (Benedict, 1938), bats (Hock, 1949) and others and has scarcely anything to do with climatic adaptation but is rather tied up with the hibernation reaction which occurs in all climates.

In birds we do not know the relation between basal metabolic rate and weight as well as in mammals. In Figure 2 our few arctic and tropical birds have been plotted upon the standard exponential curve given by Brody (1945), based on domestic birds (hens, pigeons, sparrows, canaries). The snow buntings migrate south in the winter and their high critical temperature (+ 10° C.) indicates that they were under continuous cold stress during all of the winter at Point Barrow. Nevertheless their basal metabolism was not elevated. Our two gulls were high, and normally they also migrate to warmer climates in the winter. In a series of large birds, Benedict and Fox (1927) found the highest figures for pelicans and gulls. Scholander (1940) found high figures for penguins, and many aquatic mammals are also high (Fig. 3). In Benedict and Fox a series of large birds from the New York Zoological Park includes many tropical species and they fall near the standard line, together with the temperate birds. Two of our night hawks are very low, and this is probably due to their faculty of hibernation. More material is necessary before we can tell whether the basal metabolism in birds may be adaptive to climate.

It should be pointed out that ontogenetic adaptations of the basal metabolic rate to cold have been induced experimentally in several species of mammals and birds (Gelineo, 1934, 1939; Schwabe, Emery and Griffith, 1938; Ring, 1939; Lee, 1942). The rise is generally below 20 per cent, but can get up to 50 per cent, and depends upon the length of exposure and the degree of cold. It is not clear whether the rise would be permanent. It would seem likely that in some animals at least, within reasonable limits and given enough time, the insulation would take over the whole adaptation, permitting the metabolism to drop back to normal. In any case a mere 50 per cent increase in the metabolic rate is an ineffective and expensive adjustment to a gradient which may have changed 10 times or more! We did not find a high basal metabolic rate in pigeons which had been kept for four months at Point Barrow at 0° C. to − 40° C. air temperature.[6] Dugal and Thérien (1947) found that rats and guinea pigs needed large quantities of ascorbic acid to adapt to cold, *i.e.*, to thrive and grow at a body-to-air gradient which was two to three times larger than that of the control animals. The cold-adapted animals consumed only about 40 per cent more food than the controls, however, and it therefore seems that the major factor in their cold adaptation must have been a decrease in their heat dissipation. The diminished heat loss could have been due to growth of more fur, establishment of colder and deeper tissue gradients, difference in posture, etc.

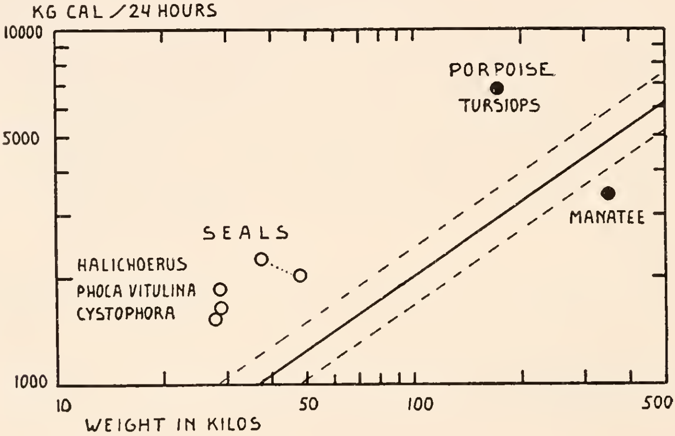


FIGURE 3. Basal metabolic rate in relation to body size in some aquatic mammals. Full drawn line is mouse to elephant curve, with 20 per cent deviation lines dotted (cf. Fig. 1). The seal records are taken from Irving, Solandt, Solandt, and Fisher (1935) and Scholander (1940), the porpoise from Irving, Scholander, and Grinnell (1941), and the manatee from Scholander and Irving (1941).

Very feeble seasonal changes of basal metabolism (± 5 per cent) have been observed in pigeons (Dontcheff and Kayser, 1934) which may, however, have been caused by factors other than temperature. In the regular and fully developed seasonal adaptation to cold which we see in our wild species, it seems likely that the insulation is normally responsible for the whole temperature adaptation. Ontogenetic changes in the basal metabolic rate of man, induced by seasons or transfer to hot or cold environment, are small and difficult to interpret (DuBois, 1936; Ames and Goldthwait, 1948); man does it mainly by insulation.

---

[6] Pigeons are known to stand considerable cold (Horvath, Folk, Craig and Fleischmann, 1948) and a number of them were kindly sent to us from Edgewood, Maryland, at the suggestion of Dr. David Bruce Dill.

THE RELATION OF COLD HARDINESS TO INSULATION AND BASAL METABOLISM

The adherence of mammals from all climates to the mouse to elephant curve holds astonishingly well in general, but there are marked exceptions. It applies only to adult animals, young ones being generally high (cf. Brody, 1945, p. 406). This undoubtedly is the explanation for the high basal rates of our very young polar bear cubs and husky pups. There are also well known examples of animals with substandard metabolic rate, notably the marmot (Benedict, 1938) and the sloth (Ozorio de Almeida and Branca de Fialho, 1924).

For all these animals, whether their metabolic rate falls on the standard line or not, heat output must balance the heat production, provided they maintain the body tem-

TABLE II

Basal metabolism of some arctic and tropical mammals and birds

The basal rate (BM) is the lowest range of the resting rates (RM) given in Figures 4, 5, and 6 of preceding paper

BM = f × RM

Species	Weight grams	f	BM kg. cal./ 24 hrs.	Species	Weight grams	f	BM kg. cal./ 24 hrs.
Arctic mammals				Arctic birds			
Dog (pups)	14,500	0.85	1,140	Gull	1,600	1.0	304
Dog (pups)	9,000	0.85	930	Raven	850	1.0	92
Polar bear (cubs)	9,300	1.00	750	Pigeon, domestic	510	1.0	33
Polar bear (cubs)	8,500	1.00	690	Pigeon, domestic	400	1.0	28
White fox	5,500	0.85	353	Pigeon, domestic	375	1.0	47
White fox	4,600	0.85	268	Pigeon, domestic	300	1.0	49
White fox	4,000	0.85	234	Pigeon, domestic	400	1.0	49
Ground squirrel	1,250	0.70	77	Alaska jay	65	1.0	22
Ground squirrel	940	0.70	72	Alaska jay	62	1.0	18
Ground squirrel	880	0.70	55	Snow bunting	35	0.75	14
Ground squirrel	880	0.70	67	Snow bunting	48	0.75	18
Ground squirrel	870	0.70	59	Snow bunting	38	0.75	13
Weasel	70	0.85	31	Snow bunting	48	0.75	18
Weasel	38	0.85	29	Snow bunting	40	0.75	17
Lemming	56	0.9	9.6	Snow bunting	40	0.75	14
Lemming	52	0.9	12	Snow bunting	40	0.75	12
Lemming	45	0.9	8.8				
Tropical mammals				Tropical birds			
Coati	5,100	1.0	335	Night hawk	43	1.0	7.7
Coati	3,200	1.0	163	Night hawk	43	1.0	7.6
Two-toed sloth	3,770	1.0	71	Night hawk	49	1.0	13
Raccoon	1,160	1.0	53	Manakin	12	1.0	6.4
Night monkey	820	1.0	48				
Jungle rat	265	1.0	39				
Marmoset	225	0.9	27				

perature constant, and we should expect that animals with the same critical gradient, living under the same climatic conditions, would show an inverse relation between the metabolic rate and the insulation according to the formula $\Delta\ TC =$ Insulation × basal metabolism. If we compare our weasels with the "standard" lemmings (Fig. 1) we see that the weasels have two to three times as high basal rate, but only half the insulation, and we may therefore say that the weasel requires less insulation *because* its metabolism is so high. Our little polar bear cubs and husky pups were observed to have much shorter fur than the adults, and evidently needed less insulation *because* of their high metabolic rate.

The sloth, with less than half the standard metabolism, has a fur nearly twice as warm as other tropical mammals (for instance, the coati) but even so its metabolism is barely enough to keep it warm, as evident from its extreme temperature sensitivity.

TABLE III

Critical gradients (ΔTC) observed from respiration curves compared with gradients calculated from measurements of fur insulation and basal metabolism (all temperatures centigrade)

Arctic species	White fox	Weasel	Lemming	Ground squirrel (Summer)
Observed ΔTC	80°	22°	20°	20°
Calculated ΔTC	68, 82, 70	40, 27	13, 13, 18	17, 18, 19

Tropical species	Coati	Jungle rat	Marmoset	Night monkey	Raccoon	Two-toed sloth	Three-toed sloth
Obs. ΔTC	15	14	13	10	9	8	—
Cal. ΔTC	13	8	11	11	5	9, 10, 7, 8	6, 6, 9, 5

It seems that another tropical mammal, the armadillo, evidently can have a basal metabolic rate as low as that of the sloth, although it is almost naked. Ozorio de Almeida and Branca de Fialho (1924) found practically normal basal rates in six specimens, and Scholander, Irving and Grinnell (1943) found only half of that rate in three specimens of the same species. Whatever the reason for this latitude in basal rate, we may observe that the armadillo is conspicuously chubby, presenting a low surface area compared with the long-armed and long-legged sloth. It is also relatively heavier due to its bone armor and the two species are not comparable in regard to microclimatic environment, for the armadillo with its burrowing habit does not live as exposed to the weather as does the arboreal sloth. Allee (1926) found four times less temperature variation at ground level in the jungle of Barro Colorado than in the canopy 86 feet above ground level, and it may be that arboreal mammals, on the whole, have warmer fur than the terrestrial forms in these parts of the tropics.

From our insulation and metabolism measurements it is theoretically possible to make an estimation of the critical gradient appropriate to the different animals, provided that the fur insulation is maximal and provided we can get a rough estimate of the surface area of the animal. If we know the rate of heat transfer through a given fur area at a one degree gradient, and we find that T times as much heat passes through the same area when the fur is on the animal, then we know that the gradient is T degrees, provided that other factors remain constant.

We know the heat transfer per unit surface area and per degree from our insulation measurements and we know the total heat loss through the basal metabolism determinations; the surface can be approximated roughly by using Meeh's formula, $0.1 \times \text{Weight}^{2/3}$, which gives the approximate surface in m^2 when weight is expressed in kg. Accordingly we find the critical gradient is equal to: $\dfrac{\text{Cal./24H/0.1 W}^{2/3}}{\text{Cal./24H/m}^2\text{/1°C.}}$.

We see from Table III that the critical gradient, as calculated from insulation and metabolism, gives a fair correlation with the observed critical temperature of the tropical and arctic mammals. The relative order is fairly well represented and the absolute magnitudes of the gradients come out surprisingly well.

Summary

Maintenance of constant body temperature in a homoiothermic animal depends upon a balance between heat production and heat dissipation, and there are consequently three possible main avenues for climatic adaptation, (1) by body-to-air gradient, (2) by heat dissipation, and (3) by metabolic rate. There is no evidence of adaptive low body temperature in arctic mammals and birds, or high body temperature in tropical mammals and birds. The body-to-air gradient can be adapted only by means of behavioral thermoregulation (nest building, avoidance of direct sunshine, etc.). With few exceptions our adult arctic and tropical mammals and birds have a basal metabolic rate that fits the standard mouse to elephant curve, *i.e.*, the basal metabolic rate is determined by an exponential relation to size, evidently fundamental to most animals, warm-blooded or not. The basal metabolic rate is consequently not influenced by such factors as temperature gradient and insulation which largely determine the heat loss, and is hence inadaptive to climate. Equally inadaptive is the body temperature, and the phylogenetic adaptation to cold or hot climate therefore has taken place only through factors that regulate the heat dissipation, notably the fur and skin insulation.

For any temperature gradient where the body temperature is maintained, the over-all insulation and the metabolic rate must be so adjusted that their product is proportional to the gradient. This is confirmed by our material inasmuch as the observed critical gradients can be approximately predicted from fur insulation and basal metabolic rate. Under the same climatic conditions there may be an inverse relation between metabolic rate and fur insulation.

Acknowledgments

We wish to acknowledge the excellent cooperation of the officers, civilians and natives of the Point Barrow camp. We are grateful for the assistance of Captain H. W. Taylor, USN, Commanding Officer, U. S. Naval Air Station, Coco Solo, Canal Zone, in placing the facilities of the Air Station at our disposal. We wish to acknowledge the inspiration and guidance of Dr. James Zetek, U. S. Department of Agriculture, Balboa, Canal Zone, during the work at the tropical laboratory, and the invaluable assistance of Mr. Walter B. Flagg and Mr. Robert H. Stapleton. We are indebted to Dr. Peter R. Morrison, University of Wisconsin, for stimulating discussion and for permission to use his weasel data. We have been fortunate in having the inspiring critical encouragement of Dr. Alfred C. Redfield, Dr. Cecil K. Drinker and Dr. Alan C. Burton during the preparation of the data.

LITERATURE CITED

Allee, W. C., 1926. Measurement of environmental factors in the tropical rain-forest of Panama. *Ecology*, **7**: 273.

Ames, A. and D. A. Goldthwait, 1948. Influence of cold climate on basal metabolism. *Rept. Quartermaster Climatic Research Lab., Environmental Protection Ser.*, No. 136.

Benedict, F. G., 1938. Vital energetics. *Carnegie Inst. Washington Publ.* No. 503.

Benedict, F. G. and E. L. Fox, 1927. The gaseous metabolism of large wild birds under aviary life. *Proc. Amer. Phil. Soc.*, **66**: 511.

Bogert, C. M., 1949. Thermoregulation in reptiles, a factor in evolution. *Evolution*, **3**: 195.

Brody, S., 1945. Bioenergetics and growth. New York.

Brody, S. and R. C. Procter, 1932. Quoted from Brody, 1945.

Burton, A. C., 1934. The application of the theory of heat flow to the study of energy metabolism. *Jour. Nutrition*, **7**: 497.

Climatological data, Alaska. Vol. **34**, 1948. U. S. Dept. Commerce Weather Bur.

Dontcheff, L. and C. Kayser, 1934. Le rythme saisonnier du métabolisme de base chez le pigeon en fonction de la température moyenne du milieu. *Ann. Physiol. Physicochim. Biol.*, **10**: 285.

DuBois, E. F., 1936. Basal metabolism in health and disease. Philadelphia.

Dugal, L. P. and M. Thérien, 1947. Ascorbic acid and acclimatization to cold environment. *Canadian Jour. Res.*, sect. E, **25**: 111.

Erikson, H., 1950. Unpublished data.

Gelineo, S., 1934. La thermogénèse des oiseaux après un séjour prolongè à différentes températures. *Compt. Rendus Soc. Biol.*, Paris, **116**: 672.

Gelineo, S., 1939. Contributions à l'étude de la thermorégulation et de la thermogénèse des hibernants. *Bull. Acad. Roy. Sci. Math. Nat. Serbe*, B, no. 5, p. 197.

Hock, R. J., 1949. Temperature and bat metabolism. Unpublished doctoral thesis, Cornell Univ.

Hoesslin, H. v., 1888. Über die Ursache der scheinbaren Abhängigkeit des Umsatzes von der Grösse der Körperoberfläche. *Arch. Anat. Physiol., Physiol. Abth.*, p. 323.

Horvath, S. M., G. E. Folk, F. N. Craig and W. Fleischmann, 1948. Survival time of various warm-blooded animals in extreme cold. *Science*, **107**: 171.

Irving, Laurence, P. F. Scholander and S. W. Grinnell, 1941. The respiration of the porpoise *Tursiops truncatus*. *Jour. Cell. Comp. Physiol.*, **17**: 145.

Irving, Laurence, P. F. Scholander and S. W. Grinnell, 1942. Experimental studies of the respiration of sloths. *Jour. Cell. Comp. Physiol.*, **20**: 189.

Irving, Laurence, O.M. Solandt, D. Y. Solandt and K. C. Fisher, 1935. The respiratory metabolism of the seal and its adjustment to diving. *Jour. Cell. Comp. Physiol.*, **7**: 137.

Kleiber, M., 1932. Body size and metabolism. *Hilgardia*, **6**: 315.

Kleiber, M., 1947. Body size and metabolic rate. *Physiol. Rev.*, **27**: 511.

Krogh, A., 1941. The comparative physiology of respiratory mechanisms. Univ. Pennsylvania Press, Philadelphia.

Lee, R. C., 1942. Heat production of the rabbit at 28°C. as affected by previous adaptation to temperatures between 10° and 31°C. *Jour. Nutrition*, **23**: 83.

Mayer, A. and G. Nichita, 1929. Sur les variations du métabolisme du lapin après exposition au froid. Variation saisonnière du métabolisme du lapin et modification de la fourrure. *Ann. Physiol. Physicochim. Biol.*, **5**: 621.

Martin, C. J., 1902. Thermal adjustment and respiratory exchange in monotremes and marsupials. *Phil. Trans. Roy. Soc. London*, B, **195**: 1.

Morrison, P. R., 1950. Unpublished data.

Ozorio de Almeida, A. and Branca de A. Fialho, 1924. Température et métabolisme du tatou (*Tatusia novemcincta*). *Compt. Rendus Soc. Biol., Paris*, **90**: 734.

Ozorio de Almeida, A. and Branca de A. Fialho, 1924. Métabolisme, température et quelques autres déterminations physiologiques faites sur le paresseux (*Bradypus tridactylus*). *Compt. Rendus Soc. Biol., Paris*, **91**: 1124.

Richet, C., 1891. De la mésure des combustions respiratoires chez les mammifères. *Arch. Physiol.*, **3**: 74.

Ring, G. C., 1939. Thyroid stimulation by cold. *Amer. Jour. Physiol.*, **125**: 244.

RUBNER, M., 1883. Über den Einfluss der Körpergrösse auf Stoff- und Kraftwechsel. *Zeitschr. Biol.*, **19**: 535.

RUBNER, M., 1924. Über die Bildung der Körpermasse im Tierreich und die Beziehung der Masse zum Energieverbrauch. *Sitzber. Preuss. Akad. Wiss.*, **17**: 217.

SCHOLANDER, P. F., 1940. Experimental investigations on the respiratory function in diving mammals and birds. *Hvalraadets Skr.*, **22**: 1. Det Norske Vidensk.-Akad. Oslo.

SCHOLANDER, P. F. AND LAURENCE IRVING, 1941. Experimental investigations on the respiration and diving of the Florida manatee. *Jour. Cell. Comp. Physiol.*, **17**: 169.

SCHOLANDER, P. F., LAURENCE IRVING AND S. W. GRINNELL, 1943. Respiration of the armadillo with possible implications as to its burrowing. *Jour. Cell. Comp. Physiol.*, **21**: 53.

SCHWABE, E. L., F. E. EMERY AND F. F. GRIFFITH, 1938. The effect of prolonged exposure to low temperature on the basal metabolism of the rat. *Jour. Nutrition*, **15**: 199.

TERROINE, E. F. AND G. DELPECH, 1931. La loi des surfaces et les vertébrés poïkilothermes. *Ann. Physiol. Physicochim. Biol.*, **7**: 341 .

TERROINE, E. F. AND S. TRAUTMANN, 1927. Influence de la température extérieure sur la production calorique des homéothermes et loi des surfaces. *Ann. Physiol. Physicochim. Biol.*, **3**: 422.

VOIT, E., 1901. Über die Grösse des Energiebedarfes der Tiere im Hungerzustande. *Zeitschr. Biol.*, **41**: 113.

WETMORE, A., 1921. A study of the body temperature of birds. *Smithsonian Misc. Coll.*, vol. 72, No. 12, p. 1.

WEYMOUTH, F. W., J. M. CRISMON, V. E. HALL, H. S. BELDING AND J. FIELD II, 1944. Total and tissue respiration in relation to body weight. A comparison of the kelp crab with other crustaceans and with mammals. *Physiol. Zool.*, **17**: 50.

WISLOCKI, G. B., 1933. Location of the testes and body temperature in mammals. *Quart. Rev. Biol.*, **8**: 385.

WISLOCKI, G. B., AND R. K. ENDERS, 1935. Body temperatures of sloths, anteaters, and armadillos. *Jour. Mammal.*, **16**: 328.

ZEUTHEN, E., 1947. Body size and metabolic rate in the animal kingdom. *Compt. Rendus Lab. Carlsberg*, vol. 26, no. 3.

REPRODUCTION OF DUDRESNAYA CRASSA HOWE[1]

WM. RANDOLPH TAYLOR

University of Michigan

During the spring of 1949 the writer undertook, at Bermuda, certain morphological studies of marine algae designed to advance the preparation of a manuscript on the tropical Atlantic algae which he has long had in hand. Several of these studies concerned themselves with Rhodophyceae. One of these in particular, *Dudresnaya,* proved to be exceedingly easy to prepare for study, and to yield preparations in which most stages seemed diagrammatically clear; yet it proved to have a very peculiar condition in the postfertilized carpogenic branch, suggesting that perhaps further development could occur either with or without demonstrable opportunity for a diploid nucleus to enter the system of connecting filaments or oöblasts. Since tetrasporangia are not known to occur in this species, though they are well known in related genera and are the expected site of meiosis, the peculiarities of the carpogenic branch are especially interesting.

This work was facilitated by a grant from funds placed at the disposal of the Bermuda Biological Station by the American Philosophical Society. For this generous aid, and for the very cordial cooperation of the former Director of the Station, Dr. D. E. S. Brown, and of his assistant under the grant, Mr. Albert J. Bernatowicz, the writer is exceedingly grateful. During the study of this material Dr. Isabella A. Abbott (Mrs. D. P. Abbott, Jr.), who had examined specimens of this plant while monographing the family to which it belonged, made very helpful suggestions regarding the traditional account of its reproductive development.

Howe (1905) described *Dudresnaya crassa* on the basis of material which he had collected in Castle Harbor, Bermuda, in July, 1900. Our conception of the plant has not been changed materially by subsequent collections. It is one of the most striking of the Bermudian endemic algae. The plants are dull in color, becoming a little brighter and stronger rose-red on drying. The size varies up to a height of 18 cm. or a little more and, since several main branches arise near the base, clumps 25 cm. in diameter are not uncommon. Ordinarily the texture is very soft, the main branches alone being firmly gelatinous. Indeed, it is often impossible to raise the plant from the water in an ordinary dip net, for it so drains through and adheres to the meshes that it cannot be removed. While others are a little more coherent, it is seldom possible to lift a large piece from the water without breaking. The close branching is irregularly radial and alternate, long and short intermixed, without significant tendency to a bilateral arrangement. The reports of such are probably due to the effects of spreading and drying such gelatinous specimens.

The diameter of the branches varies considerably, but in general the largest are not over 5 mm. and the ultimate branches are about 1.5 mm., sometimes more. The result is that the plants seem extremely bushy and rather coarse, but exceedingly soft and fragile. Reproduction is commonly very abundant. Spermatangial plants are, in general, smaller and lighter in color than cystocarpic plants; the cystocarps can be seen with a 12 × hand lens. Tetrasporangia were never seen. These plants afford the most beautiful demonstrations of carpogenic and auxiliary branch development, postfertilization fusions and oöblast development and activation of auxiliary cells ever seen by the writer. They are so easy to demonstrate that the plant is marvelously adapted to classroom use. Material preserved in formalin serves adequately, though not as well as living material, but alcohol-preserved material is very poor. Staining with dilute aqueous Congo Red, followed by a little very weak KOH, gives good results on fresh or preserved material. Dried (herbarium) material can be examined for taxonomic purposes by applying a small drop of water, immediately lightly scraping off the specimen with a sharp scalpel and transferring it to a drop of the Congo Red mixture. After a very few minutes the cells will have largely returned to normal shape and the specimen may be flattened under moderate pressure, and so suitably dispersed. Occasional refractory specimens may require stronger KOH, to as much as 1 per cent, to soften them, but are rarely satisfactory.

---

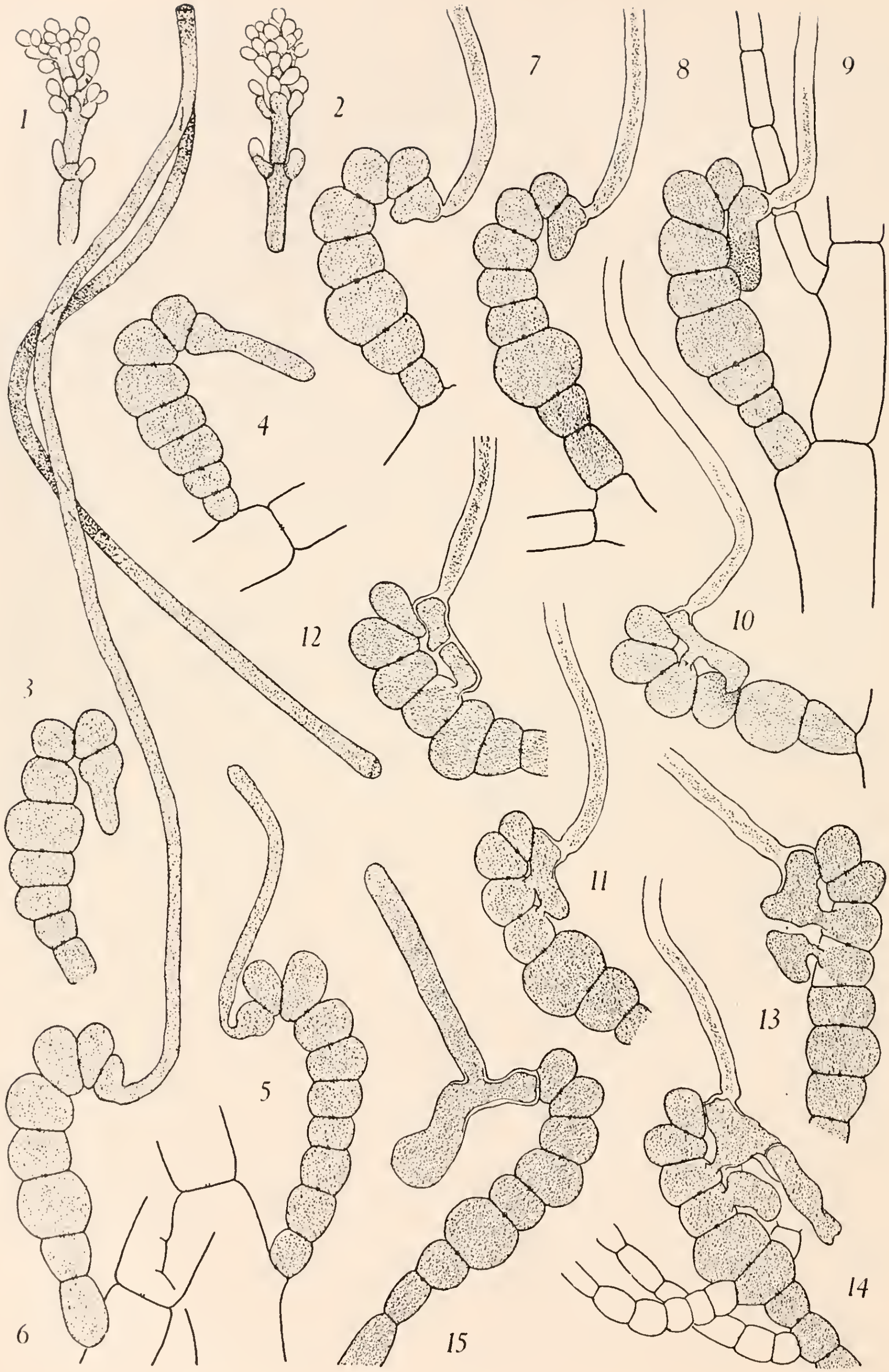
[1] Contribution No. 916 from the Department of Botany and No. 159 from the Bermuda Biological Station for Research, Inc.

The preferred habitat of *Dudresnaya crassa* seems to be on stones in bright, sheltered situations with moderate wave action and clear water, at a depth of 0.2–2.0 meters at low tide. It was common in the late spring at the Bermudas; in 1900 Howe secured it in July, but it disappeared in late summer in 1949.

There are about 30 Bermudian specimens, representing several collections, under this name in the herbarium of the New York Botanical Garden. Not all of these were microscopically examined, but a sampling of 12 showed all correctly named. In view of the situation respecting *D. bermudensis* in the Phycotheca Boreali-Americana of Collins, Holden and Setchell, numbers *1900* and *2196* were especially closely observed in all available collections. Number *1900* showed the auxiliary branch character beautifully, but number *2196* seemed a bit peculiar. It probably is poorly preserved, perhaps partly decayed before drying was completed. Nineteen group collections, some very large, were made between March and June in the course of the present study, from very diverse localities, but the plants were consistent in character.

Structually the plant shows a large-celled axis, the cells of the single row reaching 135–165 μ in diam., 300–430 μ in length, generally thin-walled, but becoming moderately thick-walled toward the base of the plant. This axis is reinforced by rhizoidal downgrowths which are 4.5–6.5 μ in diam., produced from the lower cells of the vegetative ramuli. On the axis cells toward the distal end there are borne whorls of four vegetative filaments which are repeatedly dichotomously and erectly branched, perhaps 5–6 times. The cells near the axis are pale, and subcylindrical with the outer end truncate by the two attached branches, but toward the distal end they carry more chromatophores and are slenderly subcylindrical with a slight swelling. The terminal cells are more tapering than the others and about 3.0–4.6 μ in diam. Thus the microscopic appearance is very different from that of *Acrosymphyton caribaeum* which is found in similar places at the same time of year, although it is doubtful if the two can generally be distinguished macroscopically.

Spermatangial plants are on the whole somewhat smaller than the carpogonial, and because of the abundance of pearly spermatangia on the surface often present a characteristic glaucous aspect in the water. The spermatangia are produced in

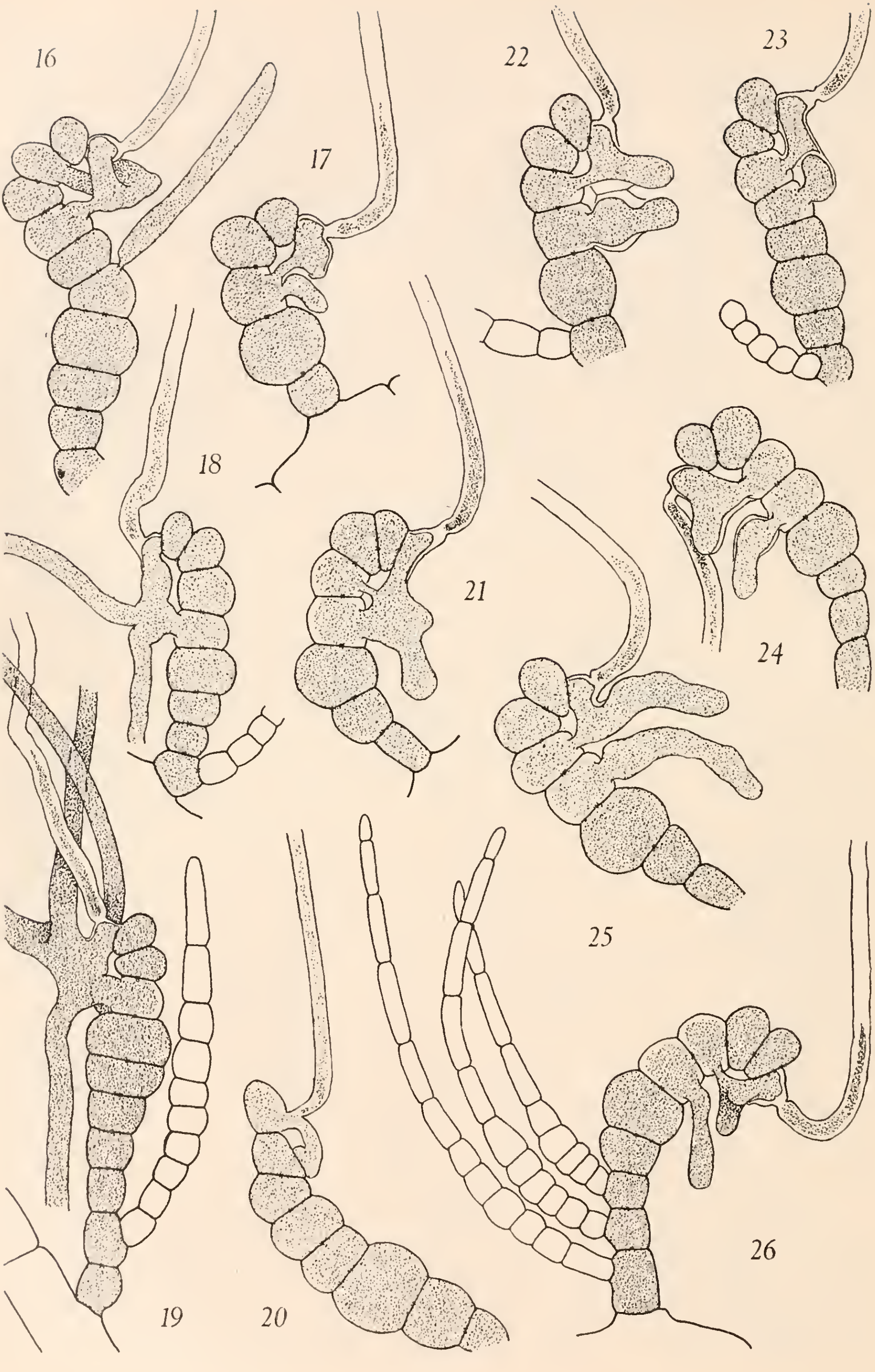


abundance on the cells toward the tips of the assimilatory or vegetative ramuli, usually opposite the stalks. They were first described by Collins and Hervey (1917).

The carpogenic branches appear laterally on the lower cells of the vegetative ramuli, alone or opposite a small branchlet, at first as a short row of a few cask-shaped cells, later reaching a length of 6–10 cells. Of these the lower may elongate a little, but the upper generally remain shorter than broad. Through the rest of this account it will be necessary to orient each observation with respect to the carpogonium, so it will be our custom to number the distal cell of the complete carpogenic branch initial, destined to become the carpogonium, as cell number 1, and those below it in succession. On this basis, then, the first distinctive change is that cells 2, 3, and 4 become wedge-shaped, causing a sharp curve, with the carpogonium rudiment close to cells 3 and 4. This rudiment then becomes flask-shaped and develops the exceedingly long, flexuous but not helical trichogyne. This, while it is at first pointed down along the carpogenic branch, quickly assumes an erect position and grows toward the surface of the plant. Mature carpogonia and many postfertilization stages were exceedingly abundant in the material available; indeed, there were so many in all stages of development that the problem was one of selection. It was quite common to find some of the lower cells of the carpogenic branch developing short simple vegetative branchlets, which generally had short cells below and more typical ones above (Figs. 14, 26, and others). The axis usually showed one cell rather larger than the others; it was usually the 5th to 7th cell from the end, but seemed to have no special function. The trichogyne often showed anomalies, particularly a simple inflation, but in some instances it forked (Figs. 15, 47). Such were probably always sterile.

After fertilization the trichogyne becomes more or less plugged near the base, leaving a remnant of cytoplasm above, and a short connecting filament is formed from the carpogonium, which proceeds to connect with a nutritive cell in the branch. The way in which this is effected does not always correspond to the classical account given for *D. verticillata* (as *D. coccinea*) by Bornet and Thuret (1876). In fact, while abundant stages and upwards of a hundred sketches recorded an ample representative series of a somewhat different story, it was with difficulty that enough examples were observed to enable preparation of illustrations to represent the expected story. To follow first the series most like this classical account, we note that the connecting filament elongates (Figs. 7–9) and makes contact with cells of the branch below it. It penetrates to first one and then another protoplast (Figs. 10, 11) by a slender tubular connection; while the proximal junction may come first (Fig. 10) it may first be complete in the distal. The carpogonium, from the beginning, retains its broad connection with the nearest point of fusion, though the trichogyne is sealed off early (Figs. 16–26). While the classical account has it that a wall cuts the connecting filament in two, so that two cells fuse separately with the two nutritive cells of the branch (Figs. 12, 23), generally no such wall appeared in the writer's

FIGURES 1–15. *Dudresnaya crassa.* Magnifications all 575 ×. Figures 1–2. Spermatangial clusters on tips of vegetative branchlets. Figures 3–6. Stages in development to maturity of the carpogonial branch. Figures 7–9. Stages in the development of the connecting filament from the carpogonium after fertilization. Figures 10–12. Junction of the connecting filament with the nutritive cells, in Figures 10 and 11 without a cross wall, and in Figure 12 with a clear one. Figures 13–14. Initiation of oöblast filaments, perhaps on each branch after activation of both cells by connecting filaments. Figure 15. Carpogenic branch with forked trichogyne.



material. It is presumed that the appropriate transfer of nuclei is effected. In cases where the connecting filament fuses with two nutritive cells without previous partition, this continuity is apparently maintained (Fig. 21). Next occurs the outgrowth of separate functional oöblasts from the fusions of each of the nutritive cells with the connecting filament. It was impossible to get any thoroughly satisfactory illustrations of this, although some were reasonably convincing (Figs. 13, 14, 22, but especially 34).

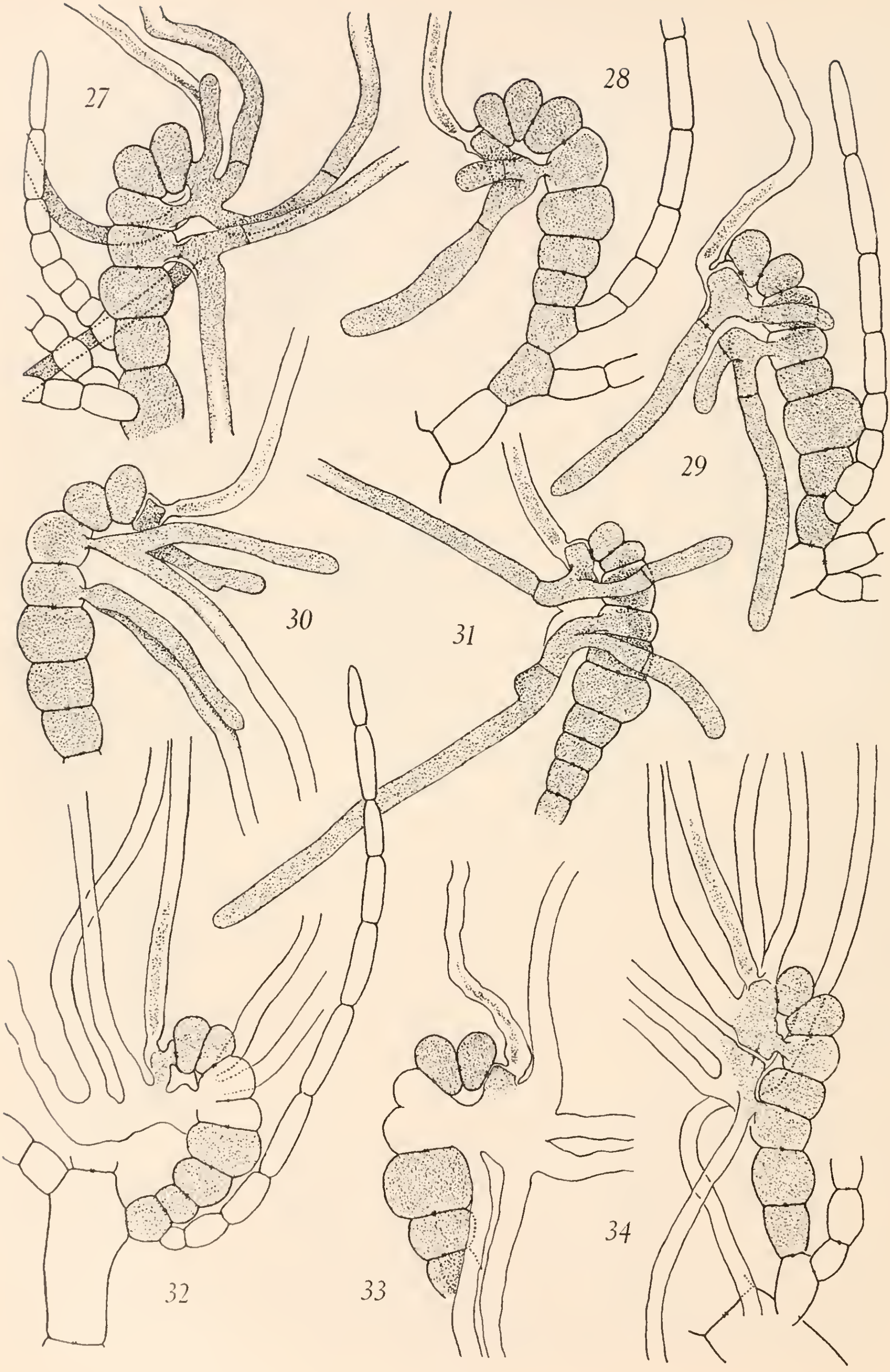
Where the connecting filament did not divide, oöblasts could arise from a single fusion structure involving all elements (Fig. 21), but this was rare. It is fairly certain that the lowest cell which could produce an oöblast was the one next above the slightly enlarged cell of the carpogenic branch.

Variations in the behavior of the connecting filament were not absent. The filament could join with various cells of the branch; perhaps the 2nd (Fig. 20), certainly the 3rd (Fig. 16), most commonly the 4th, but also the 5th (Fig. 18). Some of these were anomalous (Fig. 20), but most seemed quite normal and effective fusions. Fusions with a single cell were very common, and generally yielded fully developed oöblasts (Fig. 18).

If now we turn to a second and much commoner series of events we have a different possibility to suggest. The same initial set of figures will serve (Figs. 7–9). The connecting filament fuses with a cell in the carpogenic branch, usually the 4th cell, and the carpogonium as usual retains its wide connection. It is seldom that the oöblast emerges separately from the nutritive cell in the carpogenic branch (Fig.17). Usually a swelling develops on the connecting filament opposite its point of fusion and from this the functional oöblasts arise (Figs. 18, 25, the others). Not only this, but oöblasts emerge from the next cell below, usually the 5th cell, without any evident connection with the filament from the carpogonium. Numberless examples of this were seen. In some cases it appeared quite clear that there could have been no such fusion (Figs. 26, 30). In others the proximity of the walls of the two emergent groups of oöblasts give some cause for doubt (Fig. 22). If this is the indirect development of a functional oöblast, one may assume that it was caused by a stimulation received when the adjacent or nearby cell received the outgrowth from the carpogonium in normal fashion. There is no morphological evidence that the unconnected cell receives a carpogonium-derived nucleus through the intercellular connection or otherwise. Cytological studies were not practicable. That such cells can thus form oöblasts seems confirmed by cases like Figure 16, where a fusion with the 3rd and 5th cells (skipping the 4th) has been effected by the carpogonium and connecting

---

FIGURES 16–26. *Dudresnaya crassa.* Magnifications all 575 ×. Figure 16. Abnormal development of a fertilized carpogenic branch. Figure 17. Development of an oöblast after communication between the carpogonium and the 4th cell only. Figure 18. Development of oöblasts after communication between the carpogonium and the 5th cell only. Figure 19. Development of four oöblasts after communication between the carpogonium and the 4th cell only. Figure 20. A carpogenic branch apparently showing a connection being established between the carpogonium and the 2nd cell. Figure 21. Development of a common fusion cell after the connecting filament had joined with cells 4 and 5, and with the initial swellings for two oöblasts evident. Figure 22. Initiation of two oöblasts from cells 4 and 5. Figure 23. Carpogenic branch with clear cross-wall in the connecting filament, which has opened communication with cells 4 and 5. Figures 24–26. Three carpogenic branches with oöblasts in early stages, coming from both cells 4 and 5 in each case, but no evidence that a connecting filament reached cell 5.



filament, while from the far-removed 7th cell (skipping the 6th) a well-developed oöblast has emerged.

Quite promptly after the first oöblasts others arise successively beside them. Several may appear from each nutritive cell; how many it is difficult to say, but cases with four oöblasts from each are not unusual (Fig. 34). The reason for this obscurity lies chiefly in the increasing transparency and indefiniteness of the whole fusion structure. Walls become thicker and more gelatinous. Cell contents become less granular and less distinguishable. Stains, as far as applied, did not give much assistance. It was clear that the junctions between the connecting filament and the nutritive cells could enlarge and the walls disappear, so that these made one broad cavity (Figs. 32, 33). The cells between the carpogonium and the nutritive cells remained strikingly distinct. Walls across the oöblasts could not be anticipated in any fixed positions; though commonly appearing a little beyond the point of origin, they usually could not be found there. Intercellular connections across these walls were noted, but rarely (Fig. 29). Walls also were noted across an oöblast behind its fusion with a true auxiliary cell (Fig. 44), but not consistently.

The auxiliary cells in *Dudresnaya crassa* are intercalary, in distinctive branchlets which are formed progressively during the growth of the vegetative clusters, so that several in different stages of growth may appear in one tuft. At first these branchlets are short and blunt, not unlike young carpogenic branches, but remain straight (Fig. 35). The cells near the base soon elongate, but much more so those toward the tip, producing a characteristic long piliform extension (Figs. 36, 37).

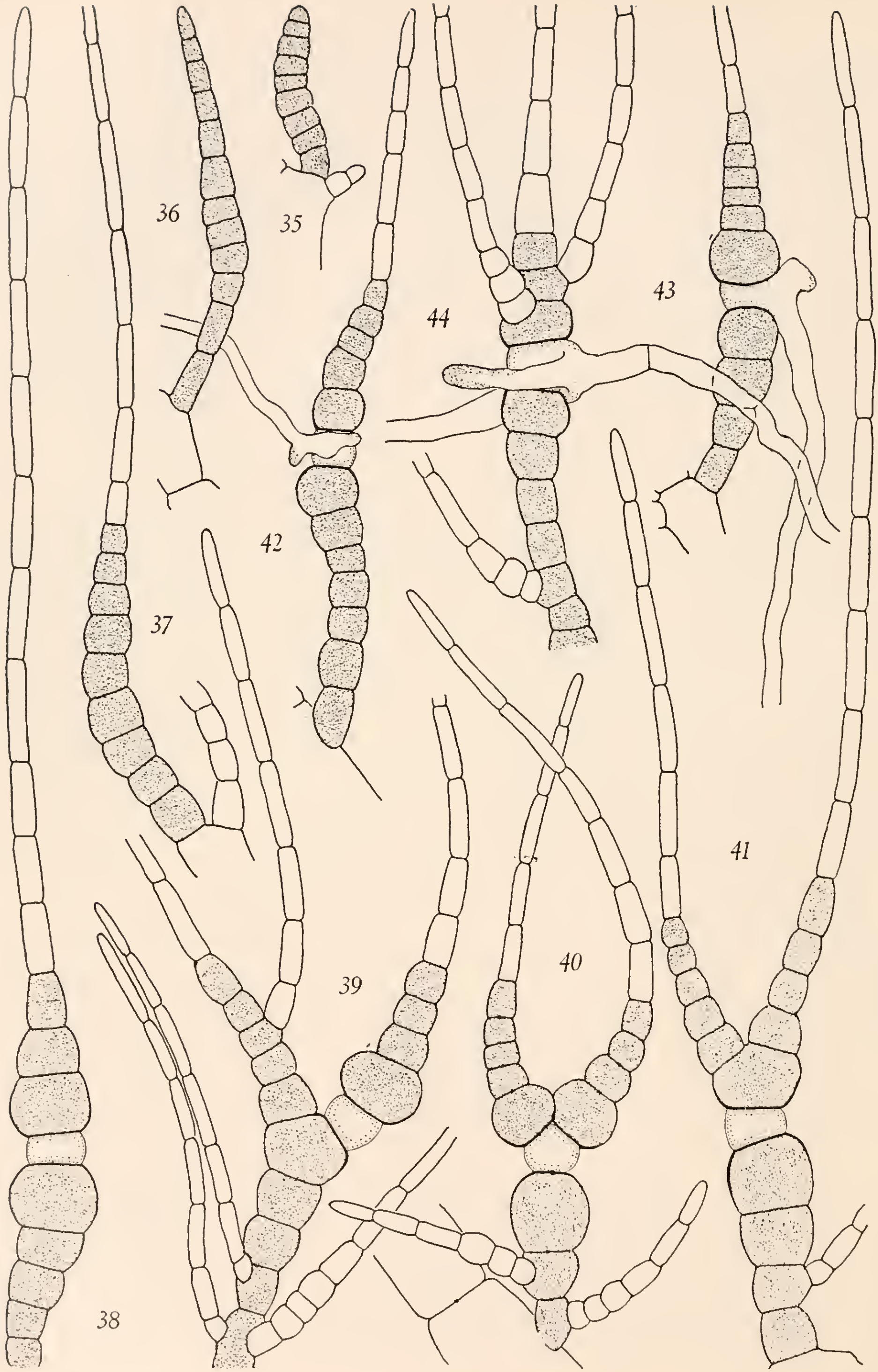
The cells toward the center, about 6–10 in number, while enlarging considerably, remain short, the length commonly less than the diameter. The final development feature is the differentiation of the auxiliary cell. This cell lags behind its immediate neighbors, which are the largest cells in the branch, appearing much smaller, rather less obviously granular in content and with a thinner wall (Figs. 38, 41). The other large cells of the branches and especially these neighbor cells seem to have an obscure, probably gelatinous thickening of the wall which shows clearly enough in stained preparations, but which is lacking on the auxiliary cell.

The auxiliary branchlet is characteristically simple, but often bears lesser lateral vegetative branchlets below, though less often above, the auxiliary cell (Figs. 39–41, 44, 48–51). Still less often, but not rarely, the axis of the auxiliary branchlet forks. This may occur at various points, and we figure examples forking on the cell below the auxiliary (Fig. 39), the cell above it (Fig. 41), and even with the auxiliary itself bearing two branches (Fig. 40).

In growing away from the carpogenic branches the oöblasts may first fuse with nearby auxiliary cells or may go some distance before opportunity for a fusion occurs. Sometimes one is favored with an exceedingly diagrammatic junction with a nearby

---

FIGURES 27–34. *Dudresnaya crassa*. Magnifications all 575 ×. Figure 27. Carpogenic branch with several oöblasts from each nutritive cell. Figure 28. Carpogenic branch with connection between carpogonium and cell 5 only, two oöblasts well established. Figures 29–31. Carpogenic branches with oöblasts well established. Figures 32, 33. Old carpogenic branches showing stages in the development of a common fusion between the carpogonium, cells 4 and 5, the remains of the connecting filament and bases of the oöblasts, which become hyaline. Figure 34. Old carpogenic branch with cells 4 and 6 acting as nutritive cells, producing eight oöblasts, their bases so approximated as to suggest that originally a connecting filament with cross-wall had been present.

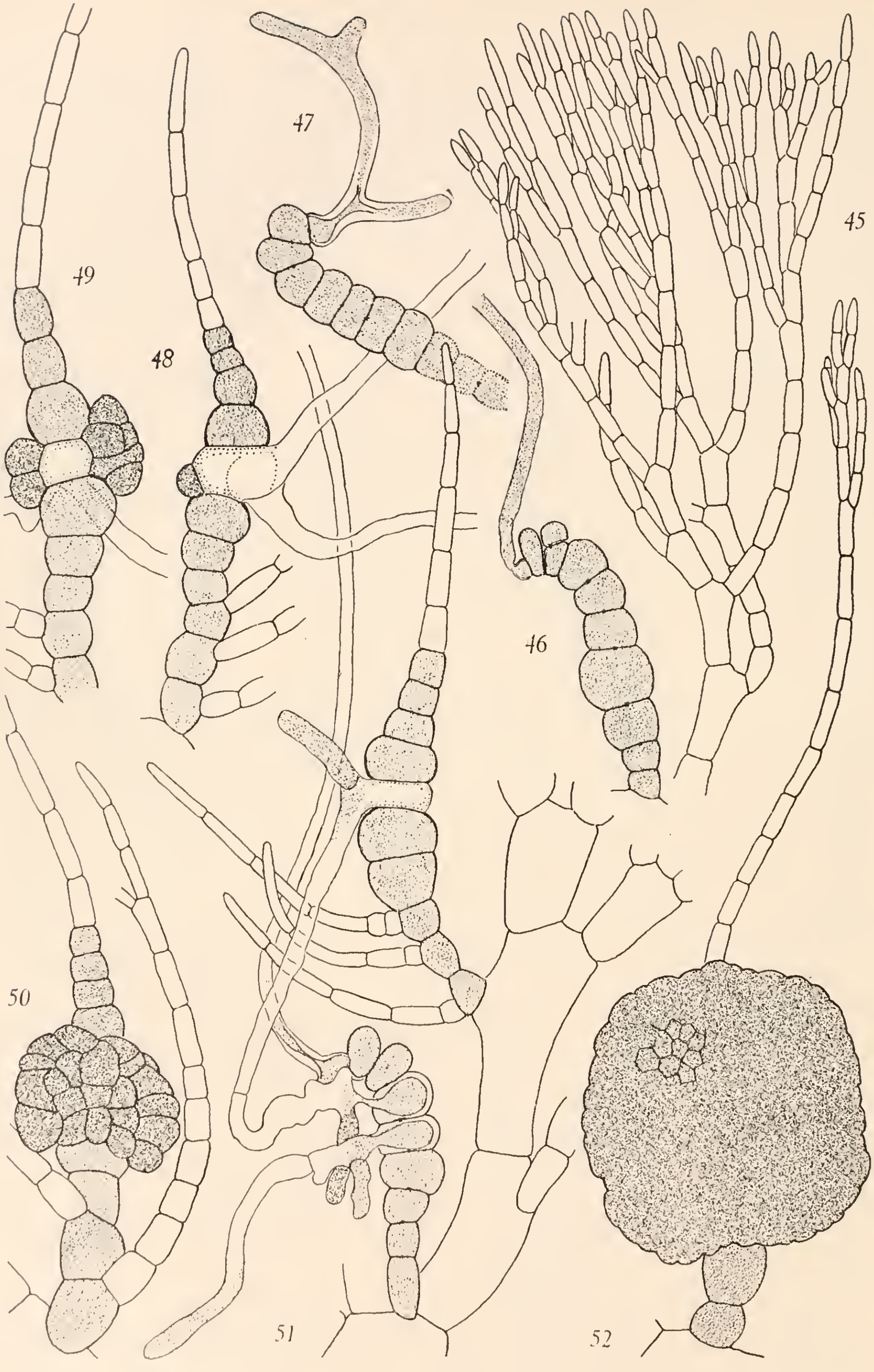


auxiliary, as when the latter is in the same branchlet cluster (Fig. 51). Sometimes it is possible to follow oöblasts from each of the nutritive cells to their respective first auxiliary fusions. In one where this occurred the distance from the 4th cell of the carpogenic branch to the auxiliary cell was 504 μ, and that from the 5th cell to its auxiliary cell was 216 μ. In another the distances were 195 μ and 357 μ, respectively, with the obvious suggestion arising that the oöblast distance from the carpogenic branch to the first point of fusion is of the order of 300 μ. It is perfectly possible to trace the oöblast from one auxiliary to the next, and this was often done, but mechanical difficulties inherent in working with material flattened by pressure preclude tracing them very far. In one that was measured, the distance from one auxiliary to the next was 396 μ.

Fusion between the oöblast and the auxiliary cells is very complete, with a general disappearance of the intervening wall (Figs. 42, 43). Immediate production of a new apex seems usual, and the new oöblast carries forward (Fig. 51). The first new oöblast may be followed by a few additional ones (Fig. 44). The cystocarp is initiated by the cutting off of a dense cell from the auxiliary obliquely opposite the oöblast connection (Fig. 48) and then, generally, a second obliquely on the other side. The result is that the round cystocarp is at first distinctly bilobed if seen from the right angle (Fig. 49), but growth soon renders this obscure (Fig. 50). The mature cystocarps are about 130–165 μ diam., short-stalked, with a long terminal filament which occasionally shows belated branching (Fig. 52). The carpospores are relatively small.

Bornet and Thuret (1867) recognized differences between their description of *Dudresnaya purpurifera* (now *Acrosymphyton*) and *Dudresnaya verticillata* (Withering) LeJolis, which they and most later writers called *D. coccinea* (C. Ag.) Crouan, but they reserved the full development of the account of that species for their beautiful "Notes Algologiques" (1876). The form of both carpogenic and auxiliary branches is clearly recognized. They saw the development of a downgrowth from the carpogonium fusing with cells in the branch, and from these fusions development of oöblasts to communicate with the auxiliary cells. As usual these are portrayed as living structures with great delicacy and accuracy. Berthold (1884) recognized the simple character of the carpogenic branch, but added nothing new. Oltmanns (1898), working with stained material, developed the nuclear account. He, even more clearly than Bornet and Thuret, recognized the formation of two cells in the extension of the carpogonium, various types of fusion between these and cells of the branch, with the later-developing communication between these and successive auxiliary cells by oöblasts. His comprehensive text (1922) has nothing new. Howe (1905) merely noted the similarity of the carpogenic and auxiliary branches in establishing the taxonomic position of *D. crassa* near *D. verticillata*. Sjöstedt (1926) restricted Dudresnaya to those species with simple carpogenic branches and intercalary auxiliary cells. Kylin (1928) confirms the account with some good new drawings. Examination of available herbarium material enabled the writer to confirm many features, but not to follow the early post-fertilization stages in the carpogenic branch.

FIGURES 35–44. *Dudresnaya crassa.* Magnifications all 500 ×. Figures 35–38. Stages in the development of a normal auxiliary branchlet. Figures 39–41. Three examples of types of branching auxiliary branchlets. Figures 42–44. Stages in the establishment of a connection between oöblasts and auxiliary cells, and continuation beyond the point of immediate fusion.



Bermudian material of *Dudresnaya crassa* has extended greatly our knowledge of variability in the reproductive apparatus. Both *D. verticillata* and *D. crassa* are considered dioecious, but a specimen of the former from Banyuls-sur-Mer showed numerous spermatangia on a cystocarpic plant, including a number on the branched tip of a cystocarp-bearing auxiliary branchlet. The carpogenic branches average one or two cells longer in *D. crassa* and the largest cells are not the nutritive cells but usually lie below these. Formation of a two-celled connecting filament from the carpogonium rarely seems to occur in *D. crassa* and the lower nutritive cell may develop an oöblast without direct connection. The auxiliary branchlets are longer (9–20 cells), the filiform tip is more pronounced in *D. crassa,* and the auxiliary cell itself distinctively smaller than its neighbors. The number of öoblasts, both from each nutritive cell and from each auxiliary cell fusion, seems greater in *D. crassa.* In cystocarp formation, Kylin (1928) suggests that the gonimoblast rudiments arise close to the attachment of the oöblast, but in *D. crassa* it appears that two are formed, to right and left on the opposite side of the auxiliary cell from the attachment. The cystocarp is at first bilobed, and with age seems larger and the spores smaller than in *D. verticillata.*

Tetrasporangial plants of *D. crassa* have not been found. They have been reported, though very rare, in *D. verticillata.* No nuclear changes have been reported at the time of the germination of the carpogonium which would suggest that meiosis took place at that point, and the same is true of carpospore formation. Doubt having been raised respecting meiosis at the close of a tetrasporophyte or at any other usual time, it becomes a question if fertilization can often be effective. If not, perhaps the formation of the second oöblast from the lower nutritive cell in the carpogenic branch need not be due to the receipt of any nucleus from the carpogonium: perhaps direct stimulation of a neighboring cell is enough to start it into growth. The nucleus it carries to the auxiliary cell will be haploid or diploid, depending on the state of the sexual plant, and so may be like that carried from the upper nutritive cell, in spite of not having been in communication with the carpogonium.

Summary

1. In *Dudresnaya crassa* the simple carpogenic branches reach a length of 6–10 cells. The auxiliary branches are much longer and taper at each end, but about 6–10 cells in the middle portion are enlarged, the functional auxiliary cell being a smaller one near the middle, and sterile branchlets are often formed laterally below.

2. Fusion of a spermatium with the trichogyne causes the development of a connection between the carpogonium and a lower cell in the branch. From this, and generally from the next subjacent cell, oöblasts are produced. There is generally

---

Figures 45–52. *Dudresnaya crassa.* Magnifications all 500 ×, except as indicated. Figure 45. Outer forkings of the vegetative or assimilatory filaments, 285 ×. Figure 46. Carpogenic branch with abnormal longitudinal division of the 3rd cell. Figure 47. Carpogenic branch with an abnormal forking trichogyne. Figures 48–50. Stages in the early development of the cystocarp. Figure 51. Unusually clear specimen showing the oöblast connection between a carpogenic branch and an auxiliary branch on the same vegetative branchlet. Figure 52. A mature cystocarp showing the persisting tip of the auxiliary filament, which in this case has branched somewhat, 240 ×.

no demonstrable connection which could deliver a diploid nucleus to the cell which produces this second oöblast.

3. The original oöblasts, and additional ones from each active cell, seem equally able to grow out, reach auxiliary cells, and initiate cystocarp formation.

LITERATURE CITED

Berthold, G., 1884. Die Cryptonemiaceen des Golfes von Neapel und der angrenzenden Meeres-Abschnitte. *Fauna und Flora des Golfes von Neapel,* **12.** Naples.

Bornet, É. and G. Thuret, 1867. Recherches sur la fecondation des Floridées. *Ann. Sci. Nat.* (ser. v, Bot.), **7**: 137–166.

Bornet, É. and G. Thuret, 1876, 1880. Notes Algologiques. **1**: 1–70; **2**: 73–196.

Collins, F. S. and A. B. Hervey, 1917. The algae of Bermuda. *Proc. Amer. Acad. Arts and Sci.,* **53**: 1–195.

Howe, M. A., 1905. Phycological Studies—II. New Chlorophyceae, new Rhodophyceae and miscellaneous notes. *Bull. Torrey Bot. Club,* **32**: 563–586.

Kylin, H., 1928. Entwickelungsgeschichtliche Florideenstudien. *Lunds Univ. Årsskr., N.F., Avd. 2,* **24** (4): 1–127.

Oltmanns, F., 1898. Zur Entwickelungsgeschichte der Florideen. *Bot. Zeit.,* **56** (6–8): 99–140.

Oltmanns, F., 1922. Morphologie und Biologie der Algen. *2te Aufl.,* **2.** Jena.

Sjöstedt, L. G., 1926. Floridean studies. *Lunds Univ. Årsskr., N. F., Avd. 2,* **22** (4): 1–95.

THE REPRODUCTIVE POTENTIAL OF A SINGLE CLONE OF PELMATOHYDRA OLIGACTIS

C. L. TURNER

Department of Biological Sciences, Northwestern University, Evanston, Illinois

INTRODUCTION

Very few metazoan animals reproduce asexually with sufficient rapidity and sustained repetition to render feasible a study of the theoretical asexual reproductive potential. After a culture of male *Pelmatohydra oligactis* had been maintained in a large aquarium for several years, during which it was observed that enormous increases in numbers by the process of budding occurred repeatedly, it appeared to the writer that it might be possible to determine the reproductive potential for males. The results are described in this paper.

Alternating periods of reproductive acceleration and depression were observed in the colony, the periods of acceleration coinciding roughly with abundance of food and the periods of depression to some extent with smaller quantities of food, but it was observed also that temporary declines in reproduction occurred even when food was abundant. Such a decline might have represented a general depression in the culture equivalent to senescence or a temporary resting stage. The only method of study which would yield results concerning a reproductive potential and the nature of the rhythms in reproduction appeared to be the method of isolating single specimens and following the reproductive history of the individuals and of their progeny. This method was pursued first by observing the increase in numbers in the progeny of a single specimen maintained in a mass and second, by separating the offspring from the parental animals as soon as they were detached and maintaining each in a separate culture.

METHODS

Methods of culturing. A substitute for pond water was obtained by running tap water into a 50 gallon aquarium in which a quantity of the Bushy Pond Weed was maintained, and drawing off and filtering the water from time to time as needed for cultures. The water varied in pH but was always slightly alkaline. Temperatures varied from 22° to 28° C. No attempt was made to measure or to control light but the rather dim light in a corner of the laboratory was accepted as suitable since the large culture had flourished under these conditions.

The food supply was maintained at a high level. Hundreds of Entomostraca were placed daily in each culture jar. The hydra fed to capacity within a few hours and killed large numbers of animals which they did not consume. All dead food animals were removed at the end of six hours. The water in the culture jars was discarded and was replaced with fresh filtered water until the next feeding period. The high level of food supply would occur occasionally in nature but the sustained daily supply would not. Since hydra reproduced very slowly, if at all, when poorly fed, the sustained and abundant supply was considered necessary to secure maximal reproduction.

The observations on preliminary mass cultures from single individuals were made on specimens maintained in half liter jars. Culture jars of 125 cc. capacity were used for single isolated specimens.

Methods of Recording. In a preliminary mass culture which was carried on for only 17 days all specimens were removed from the jar every 24 hours, counted and then replaced. All hydranths, including buds in all stages of development, were counted.

Isolation cultures were examined every 24 hours and separate records were kept for each specimen. Samples of a 36 day record of specimen 5.1.1 and a 33 day record

TABLE I

Reproductive record of specimen 5.1.1

Day	Serial numbers of buds detached	Number of buds attached	Serial numbers of attached buds	Number of new buds produced daily
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	2	1, 2	2
5	1	3	2, 3, 4	2
6	2	3	3, 4, 5	1
7	3, 4	2	5, 6	1
8	5	1	6	0
9	0	1	6	0
10	6	2	7, 8	2
11	7, 8	2	9, 10	2
12	9	1	10	0
13	0	1	10	0
14	10	0	0	0
15	0	1	11	1
16	11	3	12, 13, 14	3
17	12, 13, 14	3	15, 16, 17	3
18	15	3	16, 17, 18	1
19	0	3	16, 17, 18	0
20	16, 17, 18	1	19	1
21	0	1	19	0
22	19	0	0	0
23	0	1	20	1
24	0	4	20, 21, 22, 23	3
25	20, 21	4	22, 23, 24, 25	2
26	22, 23, 24	3	25, 26, 27	2
27	25, 26	1	27	0
28	0	2	27, 28	1
29	27	3	28, 29, 30	2
30	28, 29	3	30, 31, 32	2
31	30, 31	5	32, 33, 34, 35, 36	4
32	32, 33	3	34, 35, 36	0
33	34	3	35, 36, 37	1
34	35, 36	3	37, 38, 39	1
35	37, 38, 39	1	40	1
36	40	1	41	1

Table II

Reproductive record of specimen 5.1.40

Day	Serial numbers of buds detached	Number of buds attached	Serial numbers of attached buds	Number of new buds produced daily
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	2	1, 2	2
7	0	3	1, 2, 3	1
8	1	3	2, 3, 4	1
9	2, 3	3	4, 5, 6	2
10	4, 5	1	6	0
11	0	1	6	0
12	0	1	6	0
13	0	1	6	0
14	0	1	6	0
15	0	1	6	0
16	0	1	6	0
17	0	2	6, 7	1
18	0	3	6, 7, 8	1
19	6, 7, 8	3	9, 10, 11	3
20	9, 10, 11	2	12, 13	2
21	12, 13	4	14, 15, 16, 17	4
22	14, 15	4	16, 17, 18, 19	2
23	16, 17	4	18, 19, 20, 21	2
24	18, 19, 20	3	21, 22, 23	2
25	21, 22	2	23, 24	1
26	0	2	23, 24	0
27	23	2	24, 25	1
28	24, 25	3	26, 27, 28	3
29	26, 27	4	28, 29, 30, 31	3
30	28, 29, 30, 31	3	32, 33, 34	3
31	32, 33	2	34, 35	1
32	34, 35	1	36	1
33	0	1	36	0

of specimen 5.1.40 are shown in Tables I and II. A record was begun when a bud was detached. Thereafter the daily record for this detached bud included the appearance of new buds, the number of attached buds and serial numbers of attached buds at the time of their first appearance, and the serial numbers of these same new buds at the time of their detachment. Such a method of recording has numerous advantages. It is possible to determine by simple inspection, (1) the extent and duration of pulses of reproduction, (2) the duration of reproductive depressions, (3) the duration of the attachment periods of buds and (4) the actual rate of reproduction expressed in the production of new buds as compared to the apparent rate of reproduction which would be indicated in the numbers of attached buds. Average daily rates of reproduction were secured by considering the total number of new buds in relation to the total number of days in the period of observation. A total of 184 cultures were followed for periods ranging from 7 to 75 days and the daily average rates of reproduction were recorded.

A second and supplementary method of recording the progress of reproduction in a single clone and of numbering the individuals is shown in Figure 2. As the buds of the parent animal (5) were detached they were numbered consecutively nos. 5.1, 5.2 etc., and the numbers were placed in a clockwise series upon a surrounding circle (Gen. 1). The records of the reproduction of the detached individuals of the first generation were placed in the second circle (Gen. 2), etc. The system has the advantages of visualizing the entire output of a single animal in a single scheme, of permitting continuous expansion of the records of earlier as well as later generations and of facilitating comparisons of the outputs of members of the same generation although they are separated in time. The scheme is not intended to indicate rates of reproduction but rather extent of reproduction which may be compared in the different lines of the strain. A terminal decline in any line would be clearly indicated if it should occur. It is obvious that a high rate of reproduction would result in such large numbers as to prohibit the culturing of all specimens and the keeping of a complete record. A complete record was kept of the first two generations but selected samples were run for the later generations. Approximately 3500 specimens from 184 cultures were recorded upon the scheme shown in Figure 2. Included in the 184 cultures were 15 cultures in which the reproducing individuals died during physiological depression.

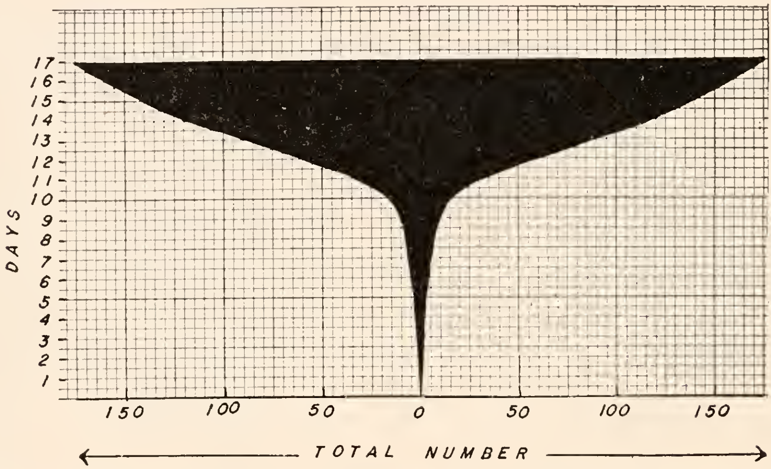


FIGURE 1. Bilateral graph showing the total number of individuals which are produced by budding by a single male specimen of *Pelmatohydra oligactis* in 17 days.

OBSERVATIONS

Single clone mass culture. The specimen which produced the mass culture shown in Figure 1 was selected from a large tank in which hydra had been maintained for several years. The specimen was large, free from gonads and buds and was in a healthy condition. The results of culturing this individual along with its progeny with an optimal food supply are shown graphically in Figure 1. Single budding occurred within 2 days and by the end of the eighth day some of the specimens in the culture were bearing one bud, some were single and a few were bearing 2 buds. Specimens bearing 4, 5 and 6 buds appeared rapidly during the next 2 days but on the last day of the 17 day period there was an increase in the number of single specimens which were not budding. Increase in numbers was very high from the tenth to the fourteenth day but the rate of increase declined slightly from the fourteenth to the seventeenth day when the culture was discontinued. The production of 350 specimens in a period of 17 days from a single specimen was a remarkable performance which was repeated by other specimens cultured under the same conditions. One of the cultures maintained for 22 days was marked by a steady decrease from the maximal rate of reproduction after the thirteenth day. The beginning of a decline in reproductive rate was not a result of an accumulation of decomposition products in the culture water since the water was changed daily. Neither was it due to crowding or a lack of food or oxygen. It was concluded from this preliminary set of observations that some internal physiological state in the strain was responsible for

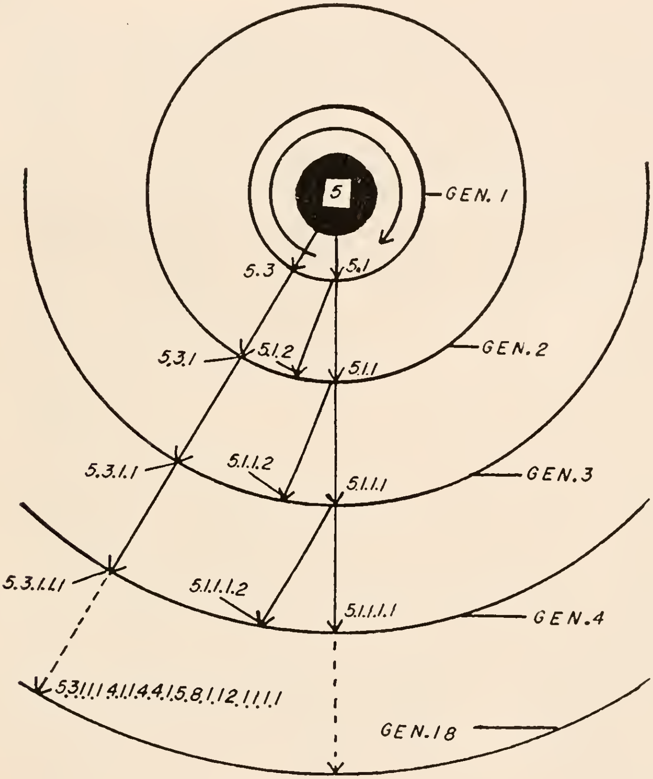


FIGURE 2. Scheme for recording the population produced by a single hydra. The scheme is capable of recording a continually expanding population and of indicating the relations of specimens by generations. The scheme does not express relations in time.

the reduction in reproductive rate but that the situation could be studied better in pedigreed individuals rather than in masses, and consequently the method of isolating single specimens as soon as they were detached was substituted for mass culturing.

Reproduction in isolated cultures. Prolonged observations upon isolated cultures were made with the primary objectives of determining the pattern in reproductive rate and of discovering whether a clone maintained under the conditions described would continue to reproduce indefinitely or would become senescent and die out. Many correlated facts were secured during the observations. It was observed that during maximal pulses of reproduction new buds might be produced by an individual at intervals of about 5 hours. The minimal time for the development and detachment of a bud was found to be about 17 hours. Multiple buds upon a parent animal are the result, in part, of the differences in time of the short intervals between new bud formations and the longer interval between the formation and the detachment of a bud. The time interval between the formation of buds is greatly extended in periods of poor food supply and also in periods of physiological depression. During periods of profound physiological depression or in complete absence of food no buds are formed. The time necessary for the growth, differentiation and detachment of a bud may be extended greatly. During the early stages of development a bud depends upon the parent animal for sources of energy but in later stages the bud feeds for itself and becomes relatively independent. If the parent animal becomes physiologically depressed during the early stages of the development of a bud, the growth and detachment of the bud may be postponed. An instance is shown in Table II (specimen 5.1.40, column 4) in which bud no. 6 arose on the tenth day but was not detached until the conclusion of the depression on the eighteenth day. If the depression in the parent animal occurs when a bud is in the later stages of its development the bud will become detached. The interval between the time of the detachment of a bud and the formation of a new bud upon the detached bud is subject also to extreme variation. When maximal reproduction is occurring the tendency to rapid formation of new buds is present in both the parent animal and the first of the new buds. Buds have been observed frequently to form buds of their own before they are detached. However, a bud detached in a period of approaching depression in the parent animal will not form buds of its own immediately and if the food supply is low the new bud will postpone its own budding until conditions are more favorable.

An examination of Tables I and II brings out the fact that there is a general correlation between the number of buds detached per day and the number of buds attached to the parent at about the same time. There is also a general correlation between the number of buds attached to the parent animal at one time and the production of new buds at the same time. The occurrence of several buds upon a parent animal is an indication of a period of rapid reproduction but it cannot be taken as an exact index of reproductive rate. For example, in Table I it is indicated that on the thirty-first day 5 attached buds were present on specimen 5.1.1 and the number of new buds formed on that day was 4. On the thirty-second day 3 attached buds were present but no new buds were formed on that day. In Table II it is shown that a bud (no. 6) was present upon the parent animal from the ninth to the eighteenth day. A casual observation of the fact would indicate that the parent animal was reproducing at a low rate when, in fact the parent animal was in a state of depression and did not form a new bud for seven days. Because of these apparent discrepancies between the number of buds attached or detached on any one day and the number of new buds formed on the same day, the rate of new bud formation was selected as the best criterion of an index for reproduction rate.

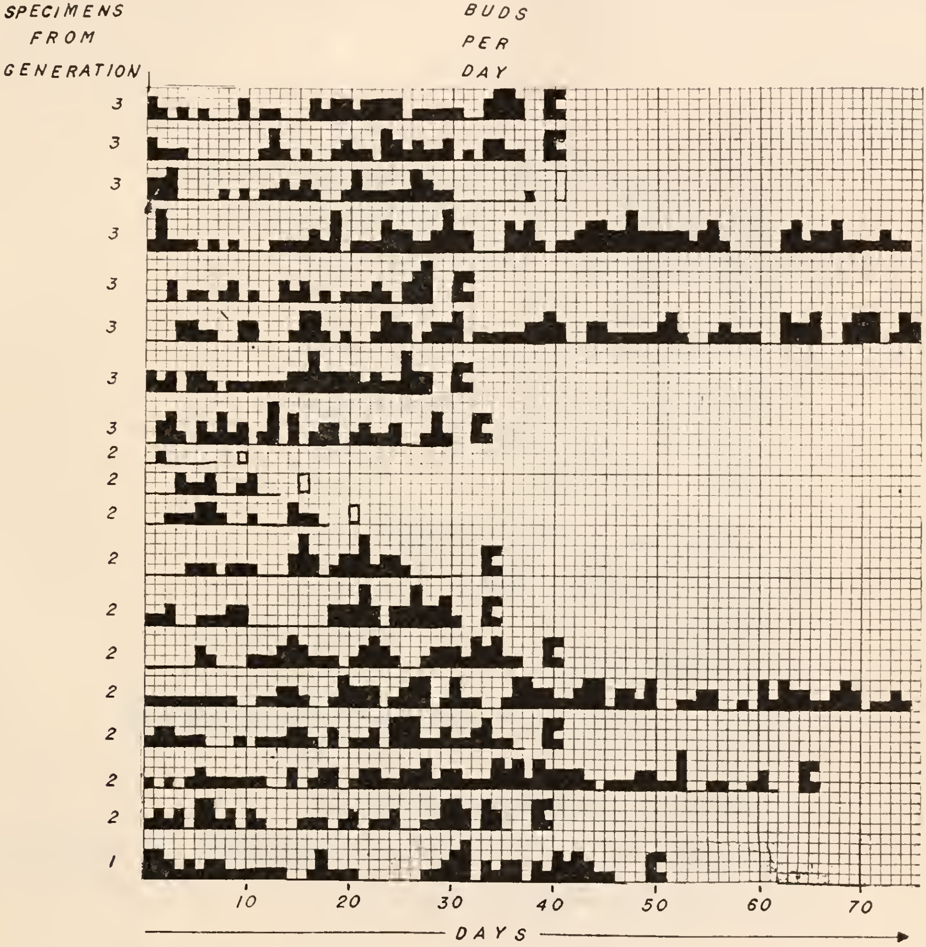


FIGURE 3. Block graph showing the daily production of buds by the single parent hydra and 18 specimens of the second and third generation. The symbol ⊏ indicates that the strain was terminated intentionally or was lost accidentally. The symbol ☐ indicates that the specimen died during a depression.

The rate of new buds produced per day by the original member of clone 5 and of 183 bud descendants representing samples from 18 generations has been plotted and 52 typical samples are shown in Figures 3, 4 and 5. Records followed by the symbol ⊏ are those which were intentionally terminated or were lost by accident. The 5 records followed by hollow rectangles indicate lines which died during physiological depression. The average rate of reproduction for each generation and for all individuals cultured is shown in Table III. The average daily production of new buds per day for all individuals cultured was 1.14 (Table III). The inclusion in the fifth and sixth generations of several cultures which died during depressions is responsible for the low rates per day shown in the Table. The reproductive rates of specimens which eventually do not survive a depression is considerably lower than the average rate for all specimens.

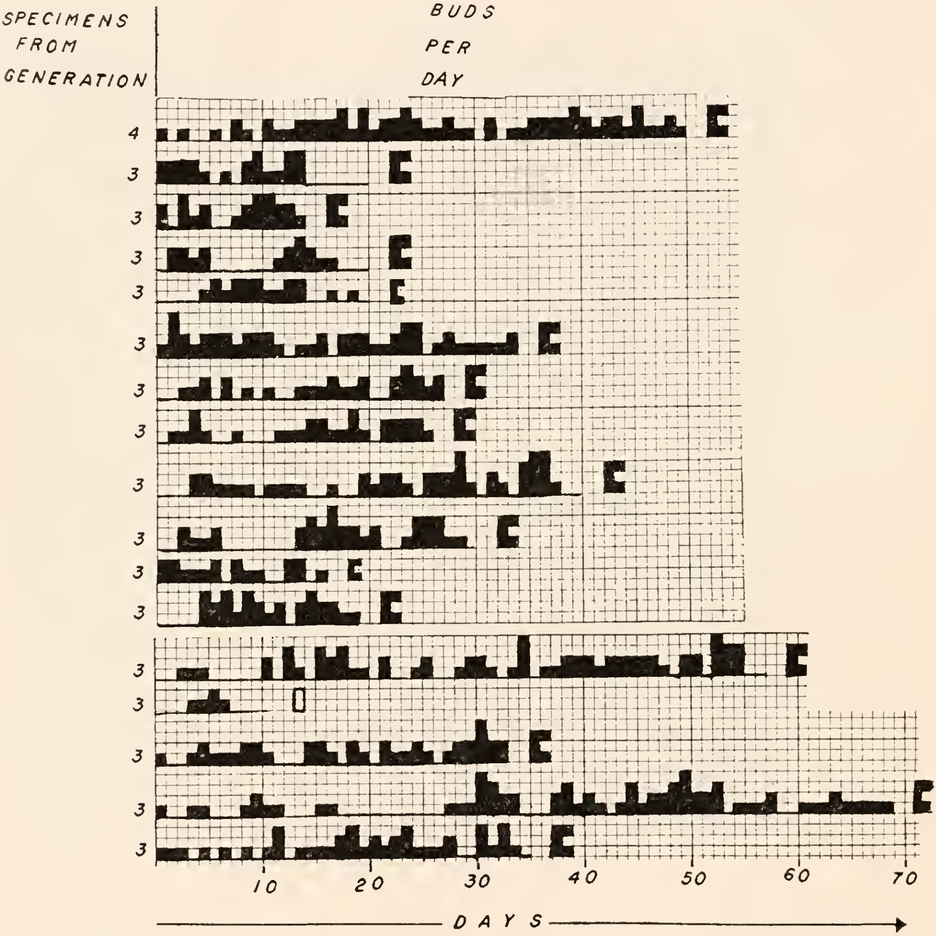


Figure 4. Block graph of 17 specimens from the third and fourth generations.

An analysis of reproduction and reproductive rates as illustrated in the graphs and Tables I and II reveals the following facts: (1) Reproduction occurs in rhythms characterized by periods of acceleration and shorter periods of rest or lowered rates per day, (2) There is no sustained reproduction at the highest level. The highest rate of reproduction for any one day is 5 and this rate is attained only twice. The longest period of sustained production of 4 or more buds per day is 2 days and 22 such periods are shown on the graphs. The longest period during which, on consecutive days, 3 or more buds are produced per day is 3 days. Eleven days marks the longest continuous period for the production of 2 or more buds per day and in no instance is there a period of more than 24 consecutive days in which one or more buds are produced. (3) In addition to the one or two day interruptions which oc-

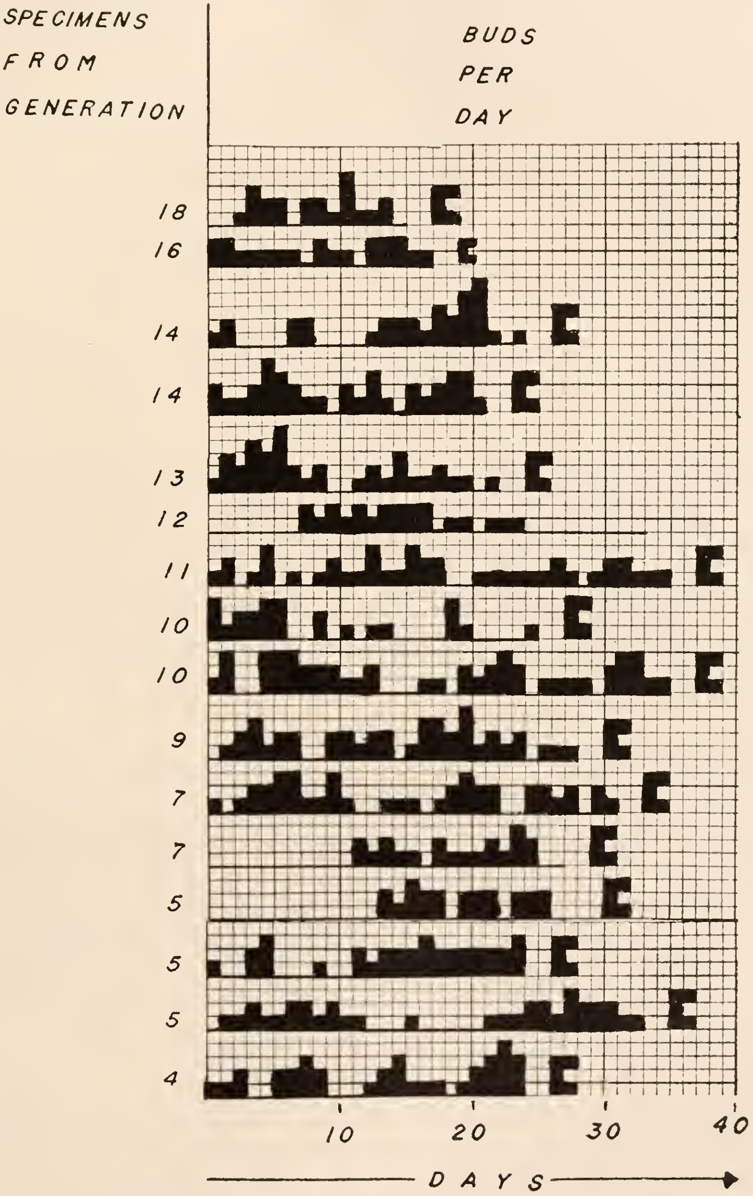


FIGURE 5. Block graph of 16 specimens from the fourth to the eighteen generations.

TABLE III

Rate of reproduction as indicated by production of new buds per day in all cultures

Generation	Average of new buds per day	Number of cultures
1	.96	22
2	1.05	50
3	1.10	42
4	1.04	22
5	.78	14
6	.65	8
7	1.00	3
8	1.15	3
9	1.18	3
10	1.27	3
11	1.05	3
12	1.31	2
13	1.43	2
14	1.24	2
15	—	—
16	1.43	2
17	1.75	2
18	1.67	1
	1.14 Gen. Av.	184 Total

cur frequently there are longer, non-reproductive periods of 3 to 12 days. These longer and less frequent intervals are periods of genuine physiological depression and they will be described later. (4) The pattern of reproduction in each individual, while irregular in number of individuals produced, is rather uniform in its repetition. There is no indication of a decline in the rate of reproduction per day or increase in the lengths of the intervals between reproductive periods. (5) No marked changes in the pattern of reproduction are apparent in different generations, from which it may be deduced that there is no general or progressive diminution in reproductive vigor in successive generations.

Periods of physiological depression. The longer periods of 3 to 12 days during which no budding occurs are marked not only by a cessation of reproduction but also by inactivity, frequently by degeneration of the tentacles and the hypostome and in a lesser number of instances by death and disintegration. The onset of this physiological state is sudden and it affects the entire animal immediately. The onset of the state is so abrupt in some instances that one or two buds still attached to the parent are caught in the depression. It may be assumed, therefore, that the onset occurred between the time of bud formation and the time of normal bud detachment. If the depression occurs while an attached bud is in a late state of development the process of detachment will continue to completion. If a bud is very young it will not become detached. It will remain attached for the duration of the depression during which growth and differentiation in the bud will not progress. The history of bud no. 6 between the ninth and the eighteenth day in specimen 5.1.40 (Table II) is a case in point. It is inferred from these observations that the state of depression originates in the parent animal but that it may extend to the bud. If the bud has established a sufficient degree of independence it will not be much affected by the depression in the parent animal. The fact that young buds do not grow and develop during depressions indicates that the state of depression extends to the processes of growth and differentiation as well as activity and reproduction.

The state of depression is set off sharply from the state which exists in partial starvation. If food is provided for a partially starved hydra the hydra reacts immediately and vigorously. If the food supply is meager the animal will produce buds slowly. If the food supply is abundant it will produce buds at an accelerated rate. On the other hand, animals in the depressed state react slowly to tactile stimulation and do not react to food at all. Cladocera, which would be captured immediately by a vigorous hydra, collide with the depressed hydra and evoke no reaction in the tentacles or nematocysts although they frequently come into contact with the tentacles and sometimes rest upon them. The starved animal possesses a full capacity for vigorous reaction, growth and reproduction while the depressed animal is incapable of vigorous reaction or growth or reproduction and in deep depression the most differentiated structures disintegrate.

Recovery from a depression is marked by a resumption of activity and feeding and, on the part of the animals that have not undergone partial disintegration, a prompt resumption of budding if the food supply is abundant. The animals which have partially disintegrated regenerate the lost parts and frequently exhibit a partial loss of morphogenetic control. The regenerated apical region is sometimes double and instances of development of supernumerary tentacles are common. In a few rare instances buds, which remained attached to the parent during the depression and were depressed themselves, on recovery from the depression remain attached to the parent but acquire sufficient independence to form a colony with the parent. Structural abnormalities which arise during depression and recovery are slowly corrected by a process of regulation. The remarkable adjustments which occur during the process of regulation will be made the subject of a separate paper. The length of a period of depression is not necessarily a mark of its severity. A depression period of 8 days may be characterized by nothing more severe than a loss of activity and reproductive output while a 3 day depression in another specimen may be accompanied by disintegration of tentacles and hypostome.

The character of the depression aside from the affects described above is not known. It might be postulated that there is a loss during rapid reproduction of some substance essential for normal metabolism, but the fact that the animals recover from the depressions and regenerate lost parts without feeding indicates rather a disturbance of arrangement of substances or processes prior to the depression and a rearrangement of the materials or processes during depression. Recovery of capacity to react to food and to metabolize the new intake would necessarily precede the actual capture and utilization of food. With the resumption of feeding and metabolizing of food a store of energy would be furnished, a part of which could be diverted to a resumption of the process of budding.

Reproductive Potential

A satisfactory formula for the theoretical reproductive potential of an animal should be capable of expressing a reproductive output for a stated period and be capable also of unlimited expansion if the factors which control reproduction are invariable. Such a formula is easy to construct in the case of a sexually reproducing animal which has a limited life span and undergoes senescence and death on the part of the parent animal. Only the progeny need be considered as the reproducers of the future. In the sexually reproducing animal the number of viable gametes, the numbers of new generations per year or season and the span of time during which the parent is able to reproduce are most important. The situation is different in an animal which reproduces asexually and in which both parent and offspring survive to reproduce on equal terms. The parent reproduces continually and long spans of time elapse between the production of the first bud and a much later one. All of the buds from a single individual belong to the same generation but the origin of the members of the single generation is spread through a long span of time. The continuity of reproduction in all generations at the same time makes it impossible to estimate the total output which constitutes one generation, or to separate the generations in time. A different method of approach must be employed if the total theoretical production is to be expressed in terms of time.

When hydra produces a mature individual by budding, and both the bud and the parent survive and continue to bud, a mathematical doubling occurs each time a bud is produced. With continual budding the doubling increases exponentially. If a rate of budding (doubling) can be established this rate can be used as the exponent of 2. If the rate is expressed in days and the daily average rate is known the following formula can be used for any stated length of time:

Reproductive output for n days = $2^n \times$ average daily rate.

The only factor which might vary would be the average daily rate of doubling. Reproduction in the original member of clone 5 and in the samples of its progeny has many minor fluctuations and long periods of depression, and some strains die out during depressions. However, an average daily rate of bud production has been obtained by including these depressed strains among the 184 samples. The records of the specimens which became depressed and eventually died have been included because each specimen was an actual or potential reproducer as long as it was alive. It has been shown also that, within the limit of 75 days at least, there is a general continuity of the daily rate in the over-all performances of individuals and of different generations. The daily average has been shown to be 1.14 under the favorable conditions described. Using 75 days as the period of time during which it is desired to express a total output, the total output under the conditions described can be expressed as follows:

$$\text{Reproductive output for 75 days} = 2^{75} \times 1.14.$$

The possibility that the potential indicated above for 75 days applies in longer periods was tested tentatively in the following procedure. Three of the oldest individuals reported for the 75 day period were observed for 48 days longer before they were accidentally lost. The average number of buds produced by these individuals for the total period of 123 days was 136. The average daily bud production was 1.10. Also two cultures of the most recently produced individuals from the 75 day period were maintained for an additional period to determine whether the production of succeeding generations would decline. The number of generations was raised to 28 in one culture and 26 in the other before they were lost accidentally. If these results are added to those described for the 75 day period it appears that there is no decline in the average daily rate of bud production or in the capacity of new buds to produce new generations, and that the formula for the total output can be used to cover a period of at least 123 days.

Discussion

Tremendous hydra populations have often been noted in natural uncontrolled situations. Some of these populations have been noted as to quantity and date with no observations as to duration. Fishing nets in Lake Erie, (Clemens, 1922) Lake Michigan (Welch and Loomis, 1924) and Lake Superior have been described as covered with hydra, in some cases for thousands of feet. Immense populations have been observed in the inland lakes of New York and Wisconsin. In some instances the inlets of water systems have been clogged with hydra. The duration of a large population in Lake Douglas, Michigan has been studied by Welch and Loomis. The large population existed during late spring and early summer but declined in late summer after the water temperature rose permanently to a point above 70° F. Large populations have been observed in other cases in late July, in November and in January, February and March in natural habitats with low temperatures at other seasons. It is indicated by these observations of hydra in natural habitats that accelerated reproduction is not seasonal except as seasonal conditions are responsible for low temperatures, food supply and dissolved oxygen in the water. In a large tank in which favorable conditions have been maintained throughout the year, the writer has observed reproductive pulses resulting in the production of thousands of specimens in each month of the year. It is clear that hydra may enter a period of reproductive acceleration at any time but, as indicated in the graphs, the periods of acceleration are sure to be followed by periods of depression. Under natural conditions a few hydra, which have survived unfavorable conditions and are in a semistarved state, will form an enormous population within two or three weeks when favorable conditions exist. If favorable conditions prevail for several weeks the extent of the population will be limited, for the most part, by the death of specimens which become depressed. During the depression specimens do not move about and the ability to undertake vertical migrations into cooler water is lost. These specimens do not feed during the depressed period and if the food supply should decline in the immediate vicinity during the depression the animals would be in an unfavorable situation on their recovery. It seems, therefore, that great populations are reduced in part by extrinsic unfavorable factors in the environment but that an important contributing factor is the intrinsic depression which inevitably follows any initial burst of reproductive acceleration.

The phenomenon of depression was first noted by Trembley in 1744 and has been studied by numerous investigators (R. Hertwig, 1906; Frischholz, 1909; Rehm, 1925; Goetsch, 1922; Grosz, 1925). Hyman (1928) has made an extensive study of the metabolic rate during depression, recovery from depression and starvation. She agrees with earlier investigators that depression may be induced by long continued feeding, extremes of temperature, insufficient oxygen and changes from pond water to tap water, but she comes to the conclusion that the primary cause is the condition of senescence during which there is a general lowering of the rate of metabolism. It follows that recovery from the depression, marked by a regeneration of lost parts and a resumption of budding, indicates a process of rejuvenescence. In none of the studies mentioned above has accelerated reproduction been emphasized as a causative factor for the depression. In the observations described by the writer depression has occurred only at the end of a period of rapid budding. It would appear that some substance necessary for normal metabolism is exhausted by rapid budding or that some toxic substance is formed incident to the rapid budding and that the lowered rate of metabolism may be caused thereby.

Senescence in highly differentiated animals is terminal and results in death. If the process of depression is a process of senescence in hydra, it is terminal for a relatively small number of specimens. The others, after partial senescence, undergo rejuvenescence and the specimens survive and resume reproduction by budding. It appears that in hydra partial senescence has been substituted for terminal senescence for most specimens and that, given favorable conditions for recovery and reproduction after depression, reproduction by budding can go on indefinitely.

SUMMARY

1. A single *Pelmatohydra oligactis*, when maintained under favorable conditions of temperature and water and given maximal amounts of food, reproduces rapidly and continually. New buds are formed every five hours when reproduction is at its height and buds may be detached in 17 hours after their first appearance.

2. Periods of maximal reproduction alternate with periods of depression during which specimens become inactive and in many instances degenerate at the apical ends. Some specimens die during the periods of depression but most of them regenerate the lost parts and resume reproduction by budding.

3. The average daily rate of bud production in 184 specimens, including those which eventually died during depressions, was 1.14.

4. There is no general decline, during a 75 day period, in reproductive vigor and rate in single specimens or in succeeding generations. During the 75 day period 18 generations were produced.

5. The total potential production by a single animal for a 75 day period can be obtained by using the average total number of doubling (budding) for the period, 75 × 1.14, as an exponent of 2.

6. Sudden reductions in population may be directly caused in natural habitats by unfavorable environmental factors, but the physiological depressions which follow rapid reproduction contribute to the decline by rendering the animals inactive and unable to accommodate themselves to changing conditions.

7. The depressions which follow rapid reproduction are basic intrinsic physiological states which may be genuine states of senescence. They may be caused by exhaustion of some substance essential to normal metabolism or to the accumulation of some substance which interferes with metabolism. Specimens appear capable of repeating the cycles of reproduction and depression indefinitely under favorable circumstances.

LITERATURE CITED

CLEMENS, W. A., 1922. Hydra in Lake Erie. *Science*, **55**: 445–446.

FRISCHHOLZ, E., 1909. Zur Biologie von *Hydra*. *Biol. Centralbl.*, **29**: 182–192, 206–215, 267–290.

GOETSCH, W., 1921. Beiträge zum Unsterblichkeitsproblem der Metazoen. I. Unsterbliche Hydren? *Biol. Centralbl.*, **41**: 374–381.

GOETSCH, W., 1922. Beiträge zum Unsterblichkeitsproblem der Metazoen. II. Lebensdauer und geschlechtliche Fortpflanzung bei Hydren. *Biol. Centralbl.*, **42**: 231–240.

Goetsch, W., 1922. Beiträge zum Unsterblichkeitsproblem der Metazoen. III. Depressionen und Lebensdauer bei Hydren. *Biol. Centralbl.*, **42**: 278–286.

Grosz, J., 1925. Versuche und Beobachtungen bei Biologie der Hydriden. *Biol. Centralbl.*, **45**: 321–352, 385–417.

Hertwig, R., 1906. Über Knospung und Geschlechtsentwickelung von *Hydra fusca*. *Biol. Centralbl.*, **26**: 489–508.

Hyman, Libbie H., 1928. Miscellaneous observations on Hydra with special reference to reproduction. *Biol. Bull.*, **54**: 65–97.

Kepner, W. R. and P. N. Jester, 1927. The reaction of Hydra to inanition. *Biol. Bull.*, **52**: 173–184.

Rehm, W., 1925. Über Depression und Reduktion bei *Hydra*. *Zeitsch. f. Morphol. u. Ökol. d. Thiere.*, **3**: 358–388.

Trembley, A., 1744. Memoires pour servir á l'histoire d'un genre de polypes d'eau donce á bras en forme de cornes. Leiden.

Welch, P. S. and H. A. Loomis, 1924. A limnological study of *Hydra oligactis* in Douglas Lake, Michigan. *Trans. Amer. Micr. Soc.*, **43**: 203–235.

OSCILLOSCOPIC AND STROBOSCOPIC ANALYSIS OF THE FLIGHT SOUNDS OF DROSOPHILA[1]

CARROLL M. WILLIAMS AND ROBERT GALAMBOS

From the Biological Laboratories and the Psycho-Acoustic Laboratory, Harvard University, Cambridge, Massachusetts

The problem of the flight sounds of insects has recently been brought to focus in the admirable monograph of Sotavalta (1947). According to this author it was in 1866 that Mühlhäuser first suggested that "the pitch of the flight-tone was exclusively caused by the number of wing-strokes per second." By matching the tone of the flight sound with that of a suitable tuning fork, one should then be in a position to assign a corresponding frequency to the wingbeat itself. This possibility was exploited by Landois (1866) and became standard practice. Unfortunately, such a procedure is complicated by various difficulties, among which is the "soprano-tenor error" whereby the flight tone is matched with a tuning fork having twice the proper frequency (Sotavalta, 1947).

Sotavalta has revived the acoustic method with notable success. Endowed with "absolute pitch," this investigator has been able to dispense with apparatus and, by the direct use of his ears and musical training, to appraise the frequency of wingbeat of free-flying insects. Thus the flight sound of insects has become a matter of contemporary interest as a quantitative approach to the study of insect flight.

It is therefore surprising that the literature contains no adequate analysis of the flight sound of any insect or definite proof that acoustic studies record the same frequency of wingbeat as do more objective methods. As Chadwick (1939) has pointed out, "the relation between the pitch of an insect's tone and the frequency of its wing-motion is still not completely understood."

Solution of this problem depends on methods whereby measurements of both the flight sound and the frequency of wingbeat may be accomplished simultaneously on the same flying insect. This objective is well within reach of present methods. The electronic stroboscope, first applied by Chadwick (1939) to the study of insect flight, permits prompt and accurate measurements of the frequency of wingbeat. Concerning the flight tone itself, Davis and Fraenkel (1940) mention its measurement by the use of a microphone and cathode ray oscilloscope, and some preliminary application of this technique has also been described by Sotavalta (1947).

In the present investigation we have applied both the stroboscopic and oscilloscopic techniques to flying insects in an attempt to establish a direct correlation between the flight sound and the frequency of wingbeat.

---

[1] The research here reported was carried out at the Psycho-Acoustic Laboratory under contract with the Office of Naval Research, U. S. Navy (Contract N5ori-76, Project NR142-201, Report PNR-96), and was aided by the Lalor Foundation of Wilmington, Delaware. Reproduction for any purpose is permitted by the U. S. Government.

MATERIAL AND METHODS

Our first experiments were performed nine years ago on the fruitfly, *Drosophila funebris*, and the mosquito, *Culex pipiens*. Although the primary problems were solved at that time, the study has been recently repeated with improved techniques of sound recording. These latter studies have been performed solely on *Drosophila funebris*.

In brief, the tip of the abdomen of each fly was attached with melted paraffin to a small wire, according to a method described previously (Williams and Chadwick, 1943). The insect was then oriented 1.5 cm. in front of a Western Electric 640 AA condenser microphone. This assembly was located in a sound-proof room from which leads passed to an adjacent room housing the recording apparatus. The latter consisted of an oscilloscope of linear sweep characteristics used in conjunction with a preamplifier and a camera of stable, adjustable film speed. A check on film speed was provided by a neon light, placed in the field of the camera and driven by a half-rectified AC potential from an audio-oscillator. The recording system had an essentially flat response from a few cycles to 5000 cycles per second.

In addition to the insect, microphone, and switch for remote control of the camera, the first room also contained a General Radio "Strobotac" for independent measurement of wingbeat frequency by the stroboscopic method. In practice it was found that the noisy discharge of the stroboscopic tube interfered with the sound picture. In order to correlate the two types of measurement, the following procedure was adopted.

Flight was induced by a puff of air. The insect's performance was tracked with the stroboscope until the animal showed constant frequency of wingbeat. The stroboscope was then switched off, the camera started, and the sound picture recorded either on continuously moving film or in a number of consecutive sweeps of the oscilloscope. Flight was terminated by touching the animal's feet with a steel rod.

For the purpose of recording the sound picture from the outset to the termination of individual flights, stroboscopic measurements were discontinued, and the flight sounds recorded on continuously moving film.

RELATION OF FLIGHT SOUND TO WINGBEAT FREQUENCY

Unlike the sound of a tuning fork, the buzz of a flying insect is by no means a pure tone. As shown in Figure 1, the exact character of the sound picture depends on the orientation of the insect with respect to the microphone. Though a sinusoidal waveform is never encountered, each orientation of the microphone yields a definite repeating pattern of fundamental wavelength.

In Table I wingbeat frequency has been estimated from the records in Figure 1 on the assumption that each repeating pattern corresponds to one cycle of wingbeat. The validity of this assumption is demonstrated by the excellent agreement between wingbeat frequencies in each flight as measured by oscilloscopic and stroboscopic techniques. This agreement persists when wingbeat frequency is increased by clipping the distal third from the tips of the wings.

These observations afford the first definite proof that, within the limits of experimental error, the fundamental frequency in the flight sound is the same as the frequency of wingbeat.

Details of the Sound Picture

As mentioned in the preceding section, the flight sound presents a waveform that depends on the orientation of the insect with respect to the microphone. In our oscilloscopic records, samples of which are illustrated in Figures 1 and 4, compression of air at the microphone is recorded as a downward deflection of the beam and rarefaction as an upward deflection. With the microphone anterior to the longitudinal axis of the insect, it will be observed in Figure 1 that the sound pattern is almost exactly the inverse of that recorded with the microphone posterior to the insect.

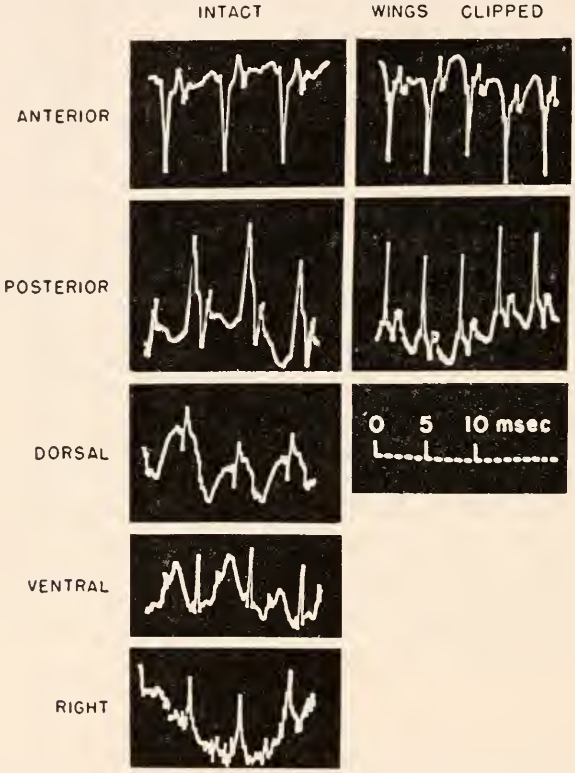


Figure 1. Sound produced by *Drosophila* during "fixed" flight. The orientation of the microphone with respect to the animal is stated at the left of each record. Compression of air at the microphone is recorded as a downward excursion. The records in the right hand column were obtained after clipping approximately one-third from the tips of the wings. Clipping the wings is seen to have little effect on the sound pattern despite the marked increase in wingbeat frequency.

As judged by its position when the insect is not flying, the baseline of zero change in air-pressure seems to lie approximately midway the vertical excursion of the oscilloscopic beam. Thus, as diagrammed in Figure 2, the microphone records a predominantly upward excursion or rarefaction anterior to the insect and a predomi-

TABLE I

Wingbeat frequency of Drosophila determined by oscilloscopic and stroboscopic techniques

	Sound frequency (beats/second)	Stroboscopic frequency (beats/second)
Intact insect:		
Head toward microphone	166	164
Abdominal end toward microphone	182	180
Dorsal side toward microphone	182	183
Ventral side toward microphone	182	186
Right side toward microphone	193	194
Wings clipped:		
Head toward microphone	250	247
Abdominal end toward microphone	270	261

nantly downward excursion or compression posteriorly. In terms of air flow this signals a net, polarized movement of air from front to rear (Demoll, 1918; Magnan, 1934).

Figure 2 attempts to correlate these pressure changes with the cycle of wingbeat. Whereas the latter is approximately equally divided into upbeat and downbeat (Magnan, 1934), the cycle of air-pressure is decidedly unsymmetrical. According to the analysis diagrammed in Figure 2, anterior rarefaction and posterior compression are present during approximately 85 per cent of each cycle of wingbeat. Only a small fraction of the wingbeat cycle, indicated by the spike in the records, produces anterior compression and posterior rarefaction. It seems necessary to conclude that polarized flow of air from front to rear is generated, not only by the downbeat of the wings, but also by a significant proportion of the upbeat.

Though the large and transient "spike" in each cycle of wingbeat cannot be accurately localized with the present data, we suspect that it corresponds to the

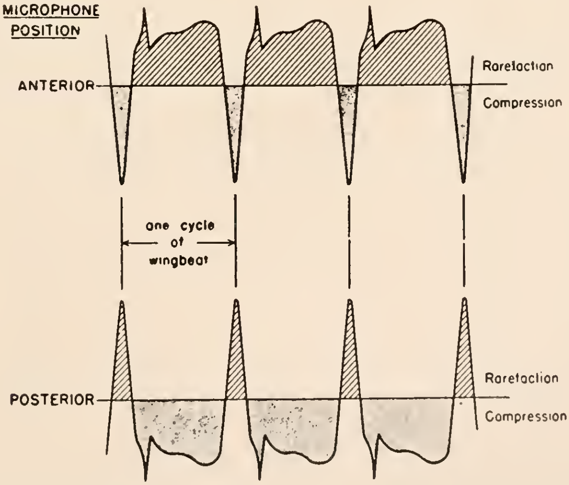


FIGURE 2. Correlation between the flight sound and the wingbeat. One cycle of sound corresponds to one cycle of wingbeat. For further explanation see text.

movement of the wings through the upper limits of their trajectory. This point could presumably be settled by studies in which the sound picture and the position of the wings were recorded simultaneously. Further study is also required to interpret the components of low amplitude that complicate the sound picture; especially is this true in regard to the rapid biphasic wave interpolated in each cycle of wingbeat (Fig. 2). Turbulences, movements of the thorax itself, and vibration of the halteres (at the same frequency but in opposite phase to that of the wings) are among the possible sources of such minor components.

Frequency of Wingbeat at the Outset and Termination of Flight

Unlike the stroboscopic method, the oscilloscope is able to track the rapid alterations in the frequency of wingbeat that occur at the outset and termination of flight. The behavior of wingbeat frequency under these conditions is recorded in Fig. 3. Two brief flights are plotted from beginning to end. In making the graphs, the total wingbeats were counted over 0.2-sec. intervals in the upper record, and over 0.5-sec. intervals in the lower one. These counts were then converted into beats per second in each case and plotted. Records of this type reveal that the early beats of the wings are accomplished at the high frequency characteristic of the entire flight.

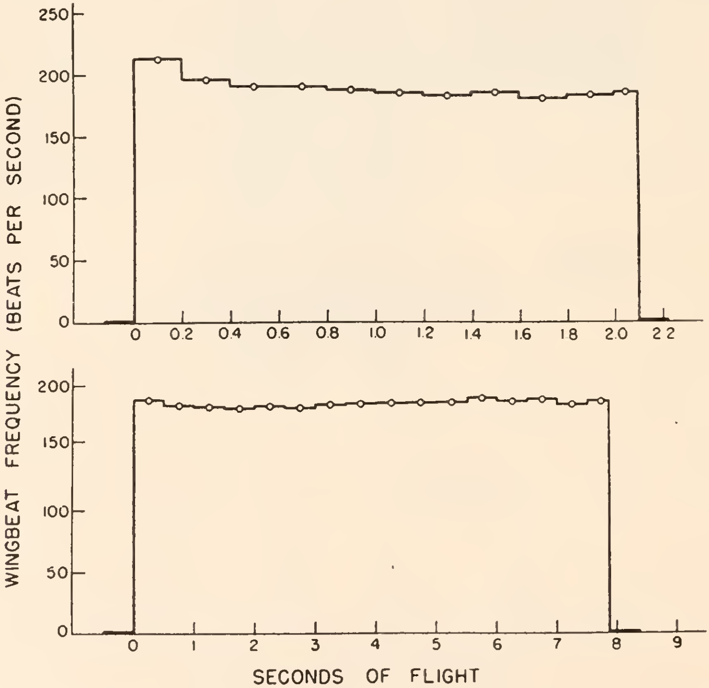


Figure 3. The frequency of wingbeat during a brief flight by each of two specimens. The frequency of wingbeat as determined from the sound records is seen to be approximately the same from the beginning to the end of each flight.

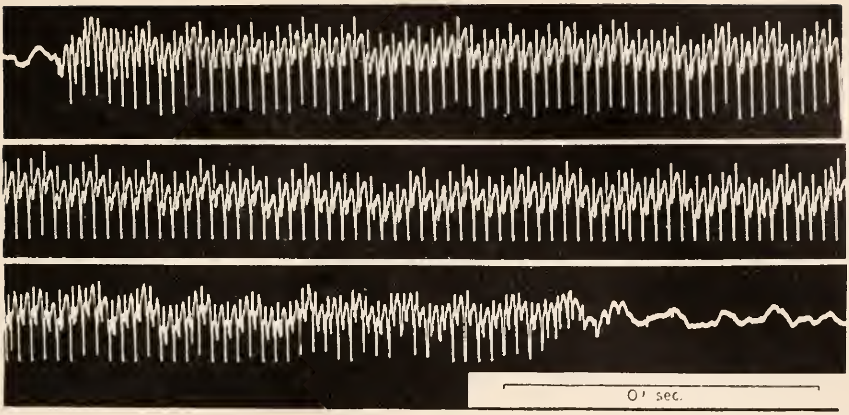


FIGURE 4. The beginning, middle, and end of a sound record of a specimen with clipped wings. The relative constancy of frequency of wingbeat, illustrated in Figure 3, is emphasized here. Irregularities at the end of the record are attributable to touching the insect with a steel rod for the purpose of terminating flight. The constant amplitude of the spikes in the middle record is an artifact due to the beam moving off the edge of the oscilloscope.

Figure 4 illustrates the sounds produced by an animal whose wings had been clipped. In this case 101 beats occur in the first 0.5 second as compared with 102 beats in the final 0.5 second. Again, the frequency of wingbeat is approximately constant from the beginning to the end of the brief flight.

DISCUSSION

Though the source of the flight sounds has long been a matter of controversy, there can be little doubt that in *Drosophila funebris* the bulk of the sound energy is the direct result of the movements of the wings. One cycle of wing motion corresponds to one cycle of flight sound. Consequently, the flight sound presents a waveform that reflects the underlying complexity of the wingbeat.

The waveforms pictured in Figure 1 are those of complex sounds. A "fundamental frequency" corresponding to the frequency of wingbeat is clear, but harmonics over a wide range are prominent and their relative strengths vary with the position of the microphone. These sounds obviously depart radically from the sinusoidal vibrations produced by tuning forks and oscillators. According to Miller and Taylor (1948), sounds very similar to these can, however, be perceived by some persons as having a pitch. The judgment is not easy to make and the accuracy with which the pure tone is matched to the complex sound is poor at best. In these facts may lie some of the subjective difficulty experienced in matching the buzz of an insect to a pure tone. Furthermore, when subjects are required to match a complex sound and a tone, a common error is to select a tone of about twice the "fundamental frequency" of the complex sound (Miller, 1950). This fact, along with the "soprano-tenor" error cited earlier, may help account for the tendency to report, from subjective flight-sound data, wing beat frequencies approximating twice the actual number (see Sotavalta, 1947, pp. 16–21). Finally, the demonstrated complexity of the insect sound should make clear the difficulties inherent in attempts to duplicate insect sounds by generating pure tones with an oscillator or tuning fork (Roth, 1948).

Analysis of the waveform, as in Figure 2, suggests that there is remarkable aerodynamic efficiency in the total cycle of wingbeat. The insect is apparently able to extract useful flight energy, not only from the downbeat of the wings, but also from the upbeat. Approximately 85 per cent of the cycle of wing motion makes positive contribution to flight; less than one-fifth of the wingbeat cycle seems to make negative contribution to the effective, polarized flow of air produced by the beating wings.

In addition to these points of interest to the aerodynamics of insect flight, the sound picture also provides useful information concerning the underlying neuromuscular mechanism. Figures 3 and 4 illustrate that during brief flights the initial and final beats of the wings are performed at the characteristic high frequency. Evidently, the mechanism that regulates the tempo of wingbeat must be fully functional at the very outset of flight.

This observation is pertinent to a theory recently proposed by Pringle (1949) to account for the unusual physiological properties of the flight muscles of flies. According to this theory,the principal features of which have been confirmed by Roeder (1950), the movement of the wings in dipterous insects depends on a remarkable myogenic mechanism. Under the general excitatory influence of the nervous system, the muscles serving the downbeat contract when stretched by the antagonistic muscles serving the upbeat. A continuation of this process leads to movement of the wings at a tempo determined largely by the loading of the flight muscles and thus, indirectly, of the wings.

Since the wing loading, under otherwise constant conditions, is fixed largely by anatomical factors, the theory would predict that the initial wingbeat be executed at the ultimate frequency. In this respect Pringle's new theory finds confirmation in the results of the present investigation.

SUMMARY

1. Oscillographic records are presented of the sounds produced by *Drosophila funebris* during "fixed" flight. Stroboscopic determinations of wingbeat frequency indicate that one cycle of wing motion corresponds to one cycle of flight sound.

2. The sounds generated during flight are complex and radically different from the simple harmonic motion of a tuning fork. Certain consequences of this fact are discussed.

3. Analysis of these sounds suggests a remarkable aerodynamic efficiency for the wingbeat cycle. The flying insect produces a polarized flow of air from front to rear during approximately 85 per cent of the wingbeat cycle.

4. During brief periods of flight the first few and the last few beats of the wings are accomplished at the high frequency characteristic of the entire flight. This fact is considered in relation to Pringle's new observations concerning the neuromuscular system of dipterous insects.

LITERATURE CITED

CHADWICK, L. E., 1939. A simple stroboscopic method for the study of insect flight. *Psyche,* **46**: 1–8.

DAVIS, R. A. AND G. FRAENKEL, 1940. The oxygen consumption of flies during flight. *J. Exp. Biol.,* **17**: 402–407.

DEMOLL, R., 1918. Der Flug der Insekten und der Vögel. Jena. 67 pp.

LANDOIS, H., 1866. Die Ton- und Stimmapparate der Insecten in anatomisch-physiologischer und akustischer Beziehung. *Z. wiss. Zool.,* **17**: 105–186.

MAGNAN, A., 1934. La locomotion chez les animaux. I. Le vol des insectes. Paris, Hermann et Cie. 186 pp.

MILLER, G. A., 1950. Personal communication.

MILLER, G. A. AND W. G. TAYLOR, 1948. The perception of repeated bursts of noise. *J. Acoust. Soc. Amer.,* **20**: 171–182.

PRINGLE, J. W. S., 1949. The excitation and contraction of the flight muscles of insects. *J. Physiol.,* **108**: 226–232.

ROEDER, K. D., 1950. Muscle potentials in various insects during flight. *Fed. Proc.,* **9**: 108.

ROTH, L. M., 1948. A study of mosquito behavior. An experimental laboratory study of the sexual behavior of *Aedes aegypti* (L.). *American Midland Nat.,* **40**: 265–352.

SOTAVALTA, O., 1947. The flight-tone (wing-stroke frequency) of insects. *Acta Ent. Fennica,* **4**: 1–117.

WILLIAMS, C. M. AND L. E. CHADWICK, 1943. Technique for stroboscopic studies of insect flight. *Science,* **98**: 522–524.

PAPERS PRESENTED AT THE MEETING OF THE SOCIETY OF GENERAL PHYSIOLOGISTS

JUNE 21–23, 1950

The mechanism of accumulation. W. J. V. OSTERHOUT.

When a compound enters a living cell, until its inside activity becomes greater than its outside activity, work must be performed to bring this about. Such a compound may be said to accumulate. (When a single ionic species reaches a higher activity inside than outside without performance of work, as in the Donnan equilibrium, this is not accumulation as here defined.)

Experiments indicate that free ions cannot pass through the nonaqueous layer which covers the protoplasm except very slowly, but by combining at its outer surface with organic carrier molecules they can move inward much more rapidly and reach the aqueous protoplasm in contact with the inner surface of the nonaqueous layer.

Certain assumptions are made as follows. On reaching the inner surface of the nonaqueous layer, metabolic processes alter or decompose the carrier molecules so that the ions are set free. The carrier molecules which have not combined suffer the same fate so that at the inner surface of the nonaqueous layer there are practically no carrier molecules to transport ions outward. Thus the free ions are trapped in the protoplasm since they cannot pass out except very slowly.

The carrier molecules are formed in the nonaqueous layer where they concentrate at the outer surface, either because they are formed there (as is the case with cellulose and a variety of organic molecules in plant cells) or because they collect there . . . since they lower surface tension. In the latter case the surface tension is altered when the carrier molecules combine with the ions which facilitates their inward passage. In any case, they will move inward after combining since there will be an inwardly directed concentration gradient.

If we adopt this point of view we can suggest answers to some important questions such as the following:

1. Why accumulation is confined to electrolytes. This is evident since only ions will be trapped.
2. Why ions appear to penetrate against a gradient. Actually there is no such penetration since the ions enter in combination with carrier molecules. The energy needed to raise the activity of entering compounds is furnished by the reactions involved in the process of accumulation.
3. Why, in absence of injury, ions do not come out when the cell is placed in distilled water. Presumably the outgoing free ions will combine at the outer surface with carrier molecules and then move inward in the same way as ions coming from without.
4. Why the relative rate of penetration falls off as the external concentration increases. Since the concentration of carrier molecules does not increase, the rate of entrance approaches a limit.
5. Why it is not necessary for anions and cations to enter in equal numbers. When, for example, K^+ unites with a carrier molecule HR we assume that H^+ is exchanged for K^+ so that electrical neutrality is preserved. When Cl^- unites with the carrier molecule ROH we assume that OH^- is given off and RCl is formed. These reactions may take place independently.
6. Why accumulation is promoted by constructive metabolism which is needed to build up the organic carrier molecules and by destructive metabolism which brings about their alteration or decomposition.
7. Why measuring the mobilities of ions in the outer protoplasmic surface does not enable us to predict the relative rate of entrance of ions. This is to be expected if ions enter by combining with molecules at the surface. Only if K^+ is able to combine preferentially will it accumulate preferentially.

8. Why ions may come out in anoxia and at low temperatures. Presumably these conditions depress the formation of carrier molecules and their decomposition in the protoplasm more than the outgo of ions so that relatively more come out.

The role of growth and metabolism in ion accumulation of plant cells. F. C. STEWARD AND F. K. MILLAR.

The nature of the relationship between the vital properties of plant cells and their ability to accumulate salts in their vacuoles was reviewed. Earlier work with storage organs has led to the view that the ability to accumulate salts is characteristic of cells which can divide, synthesize protein and have a high rate of aerobic respiration (*Tr. Faraday Soc.*, 1937, **33**: 1006 and *Ann. Rev. Biochem.*, 1935, **4**: 519).

To elucidate the relationships between metabolic properties and salt intake, new techniques were described. By the use of plant tissue cultures, it is now possible to investigate rapidly proliferating cells in contrast with cells which, though they metabolize actively, do not grow.

The system devised (*Nature*, 1949, **163**: 920 and *Science*, 1948, **108**: 655) consists of minute cylinders of the secondary phloem of the carrot root. These discs cannot grow though supplied with a seemingly complete organic and inorganic medium. However, when supplied with a factor contained in coconut milk the tissue increases rapidly some 60–80 times in about 20 days.

Apparatus for the controlled growth of these tissue cultures and the smooth growth curve were illustrated. By treating both the proliferating and non-proliferating tissue with radioactive isotopes (Cs^{137}) ion uptake has been investigated with time.

The outstanding difference between dividing and non-dividing tissue became apparent when it was realized that (1) growing tissue took up Cs^{137} *pari passu* with growth; (2) tissue which was not *externally* growing took up Cs^{137} in a similar manner with time. These two cases of uptake, however, seemed to be different.

In the growing culture, absorption of Cs^{137} was uninfluenced by addition of a large excess of carrier Cs. Thus Cs absorbed was directly proportional to the first power of the external concentration.

In the non-growing culture, however, the Cs^{137}detected in the tissue was suppressed by addition of carrier Cs since the "absorption ratio" decreases with greater concentrations in the external solutions.

The interpretation given these facts was that there are two distinct ways in which plant cells accumulate salts. Actively metabolizing, extending cells which do not divide (cultures in absence of coconut milk) absorb by the familiar mechanism of salt accumulation in the vacuole.

Rapidly dividing cells in presence of coconut milk, however, appear to bind their Cs^{137} proportionally to the external concentration and this binding is presumably cytoplasmic.

The significance of these observations for the further investigation of the problem was indicated.

Stress and the human adrenal cortex, especially in relation to potassium metabolism. HUDSON HOAGLAND. No abstract submitted.

Potassium movement in relation to drug and ion action in nerve. ABRAHAM M. SHANES.[1]

Earlier studies (*J. Gen. Physiol.*, 1949, **33**: 75) on the behavior of the resting potential during anoxia and stimulation as modified by metabolic inhibitors, substrates, ions and stabilizing and unstabilizing drugs, have led to the conclusion that fluctuations in the polarization level of both vertebrate and invertebrate nerve are due primarily to alterations in the potassium concentration at the surface of the fibers.

Potassium movement under the same experimental conditions has now been studied directly by flame spectrophotometric analyses of small volumes of solution brought successively in contact with the nerves. The results confirm the predictions based on the indirect observations and thereby establish the causal relation between potassium and bioelectrical changes.

---

[1] Supported in part by research grants from the National Institutes of Health, U. S. Public Health Service, and from the American Philosophical Society.

Thus, in crab (*Libinia emarginata*) and frog (*Rana pipiens*) fibers, anoxia causes a potassium release which is retarded by glucose; 1 mM iodoacetate has been found to counteract the glucose effect. Stimulation causes the well-known release of potassium. In addition, as anticipated from the large, long positive after-potential in crab nerve and from the corresponding post-anoxic over-shooting of the resting potential in both crab and frog nerve, potassium is taken up from the medium following stimulation and anoxia; the removal of the vertebrate sheath is extremely important in these experiments.

Additional details are now available for frog tissue: Cocaine (0.1%) retards the escape of potassium during anoxia, while low veratrine concentrations (0.2 to 0.5 mg.%) increase it three- to four-fold. Higher veratrine concentrations (1 to 2 mg.%), which cause depolarization directly, cause an escape of potassium which, like the electrical effect, can be prevented with cocaine.

A forty-fold decrease in the sodium content of the medium reduces the potassium escape during anoxia 30% and seriously impairs post-anoxic recovery. In Ringer's containing only 2 mM sodium—with choline or sucrose to make up the osmotic deficit—potassium release during oxygen lack is unaccompanied by an uptake of sodium, although in normal Ringer's roughly equivalent amounts of sodium enter.

The identity of the concentrations of the experimental agents which alter potassium lability and excitability, conduction, and the resting potential, suggests a common rôle for potassium movement in relation to these phenomena. In any event it appears likely that the establishment of additional details concerning the potassium shifts under a variety of experimental conditions will serve to clarify the basic mechanisms concerned with nerve functioning as well as with the mode of action of drugs and ions.

Ions and muscular contraction. A. SZENT-GYORGYI. No abstract submitted.

Potassium and sodium exchanges in human red cells. ERIC PONDER. No abstract submitted.

The disturbance by X-rays of selective potassium accumulation in human erythrocytes. C. W. SHEPPARD.

The maintenance of osmotic stability and the specific potassium accumulation of cells represents a fertile field for radiobiological research, the mammalian erythrocyte being a particularly advantageous material. When freshly drawn heparinized human whole blood is exposed to X-rays (54,000 r maximum) and then equilibrated under a controlled atmosphere at 24° C. or 38° C., (*Biol. Bull.*, 1948, **95**: 283), potassium is progressively lost from the cells and quantitatively replaced by sodium with little if any osmotic disturbance. The mean rate of loss at 20,000 r and 24° C. is about 1% of the initial cell potassium per hour and doubles approximately for every 20,000 r increase. The effect is accentuated by storage at low temperature (5° C.) following irradiation. By labeling the plasma potassium with K^{42} it is shown that although radiation slightly depresses the rate of entrance of potassium into the cells the reverse rate from cells to plasma is greatly accelerated.

Studies of normal cells with Na^{24} have shown that about half of the low intracellular sodium exchanges rapidly with that of the plasma sodium, the remainder exchanging very slowly. If cells are allowed to replace their potassium with sodium following a period of post-irradiation equilibration the rapidly exchanging sodium fraction increases in size, suggesting that this fraction is ionic sodium.

The interpretation of isotope experiments in systems of compartments is aided by the use of an electrical analogy in which the amount of material in a compartment is replaced by the capacitance of a condenser, the specific activity by the electrical charge and the exchange rate by the conductance of a resistor. Investigation of the electrical properties of an analogue to a system of four peripheral compartments surrounding a central one, shows that often the peripheral compartments may be replaced by a single equivalent substitute.

Studies on the permeability of human erythrocytes to potassium. I. M. Taylor and J. M. Weller.

In vitro studies have shown (*J. Gen. Physiol.*, 1950, **33**: 691) that the potassium ions of human red blood cells exchange with the plasma potassium ions at a constant rate, amounting to 1.5 millimoles per hour per liter of cells at 37° C. The temperature coefficient (Q_{10}) for the exchange rate is 2.2.

Observations have been made on the nature of the leakage of potassium from human red cells in the presence of fluoride and in the absence of glucose.

Studies have been made of the effect of cholinesterase and choline acetylase inhibitors. Both cause a breakdown of the potassium concentration gradient of the human red cell. Cholinesterase inhibitors decrease the rate of uptake of potassium from the plasma. Choline acetylase inhibitors increase the rate at which potassium leaves the cells.

Some ionic exchanges and ionic equilibria of the erythrocyte. M. H. Jacobs.

The erythrocyte is a particularly favorable cell for the study of this problem (a) because it shows two distinct types of permeability to ions, (b) because its ionic permeability can be experimentally modified in several ways, and (c) because its ionic exchanges are frequently accompanied by characteristic osmotic volume changes that can be followed quantitatively and recorded in detail by photographic methods.

The permeability of the erythrocyte to cations is similar to that of many other known cells in being low in degree and in involving an expenditure of energy by the cell to bring about ionic distributions that would not otherwise occur. Permeability to anions, on the other hand, is of a degree so high as to be unique among cells, is purely passive in character, and always proceeds in the direction of a predictable state of equilibrium.

The exchanges of many anions can be greatly accelerated by the proper use of the CO_2-bicarbonate-carbonic anhydrase system, and enormously and specifically retarded by low concentrations of tannic acid. Permeability to cations can be increased to an extent that can be regulated over wide limits, and passive diffusion can be made to take the place of active transport, by appropriate treatment of the cells with 5 or 6 per cent butyl alcohol.

Calculations have been made of the theoretical osmotic and ionic equilibria, in solutions containing any desired quantities of NaCl and sucrose, of hypothetical cells having the approximate chemical composition of the erythrocyte and each of the types of permeability so far experimentally realized. When compared with the theoretical predictions for such cells, the observed osmotic volume changes of both normal and modified erythrocytes show a very satisfactory semi-quantitative agreement, which extends even to unexpected details of the predictions.

The effect of physostigmine and acetyl choline on the permeability of erythrocytes. Margaret E. Greig.

Evidence for a relationship between the activity of cholinesterase and the maintenance of selective permeability of erythrocytes as well as certain other tissues is as follows:

1. Washed erythrocytes (dog, cat, human, beef, rabbit) which are actively metabolizing acetyl choline remain intact and maintain their original ion composition for a much longer period of time than do similar erythrocytes in the absence of substrate (acetyl choline).
2. When the cholinesterase activity of erythrocytes is inhibited by physostigmine (which is considered to be a specific inhibitor of this enzyme) changes in permeability occur at rates similar to those observed in cells without substrate; the greater the inhibition of cholinesterase, the greater the permeability to sodium, potassium and hemoglobin.
3. Substrates which are metabolized at different rates by cholinesterase had different abilities to maintain the cell intact, those which were metabolized most rapidly being most effective in preventing hemolysis.
4. A correlation between cholinesterase activity and permeability of the hemoencephalic barrier of the frog to acid fuchsin was apparent when it was found that the time of onset of convulsions caused by acid fuchsin was decreased from an average of 12.6 hours for dye alone to thirty-four minutes for those frogs in which physostigmine was administered together with acid fuchsin.

5. Physostigmine also caused local anesthetics, which had no surface activity but were infiltration anesthetics, to produce anesthesia when applied to the rabbit's cornea. There was found to be a parallelism between ability to produce surface anesthesia and ability to inhibit cholinesterase, thus indicating again that by inhibiting this enzyme the membrane is made more permeable.

6. Physostigmine decreased the lag exhibited by certain anesthetics. In the case of barbital the time of onset of anesthesia was reduced from twenty-seven minutes to eleven minutes in mice by the simultaneous administration of physostigmine. For chloralose the lag was reduced from twenty-one minutes to nine minutes. These results would indicate that physostigmine which inhibits cholinesterase increases the permeability of the blood brain barrier to anesthetics.

It thus appears that the integrity of the cholinesterase system may play an important role in cell behavior.

The role of potassium in the metabolism of E. coli. R. B. ROBERTS. No abstract submitted.

Electrolytes, metabolism and the muscle membrane potential. GILBERT LING AND RALPH GERARD. No abstract submitted.

Calcium and protoplasmic clotting. L. V. HEILBRUNN. No abstract submitted.

The influence of carbohydrates and insulin on the potassium content of leucocytes and muscle. J. F. MANERY, K. C. FISHER, D. R. H. GOURLEY, D. L. WILSON AND M. F. J. TAYLOR.

Rabbit leucocytes obtained and analysed by methods previously described (*J. C. C. P.*, 1949, **34**: 493) were shown to be relatively permeable to potassium. They readily lost most of their potassium in potassium-free Ringer's solutions and rapidly exchanged it for K^{42}. In the 45-minute centrifugation period required for packing, the specific activity of the cells was 32% of that of the fluid, while after aeration for 54 minutes plus 45 minutes centrifugation time this value was 104%.

The effect of glucose on the potassium content of leucocytes was studied. A suspension of cells was divided into two lots; one lot was exposed to a glucose-free Ringer's solution and one to a similar medium containing 160 mgm. % glucose. These suspensions were aerated at 37°C. for periods of time varying from 25 to 90 minutes. In each case the analysis of the packed cells showed that those in glucose-Ringer's solution had a higher potassium content than those in a glucose-free medium; the difference increased as the period of exposure was lengthened. Sodium appeared to exchange for potassium since a reciprocal relation was demonstrated and the cellular water concentration did not vary.

Paired frog muscles were allowed to respire in an atmosphere of oxygen in Warburg respirometers for 6 hours. At the end of the second hour experimental agents in Ringer's solution were tipped from the onset on to one member of the pair while an equivalent volume of Ringer's solution was tipped on to the control muscle. The final concentration of insulin was 0.01 units per ml. and of lactate 0.005 M. Analyses of both muscles and fluids for potassium showed conclusively that insulin caused muscles to take up potassium in the presence of lactate. Iodoacetate (0.00025 M) abolished this influence of insulin on the potassium uptake. In general the conditions under which potassium uptake occurred likewise stimulated oxygen consumption.

Studies on carbohydrate metabolism in mammalian muscle. CLAUDE A. VILLEE, VIVIEN WHITE AND A. BAIRD HASTINGS.[1]

Using techniques described previously (*J. Biol. Chem.*, **179**: 673) rat hemidiaphragms were incubated in duplicate Warburg vessels in a medium containing glucose, 11.1 mM/L., and pyruvate, 10 mM/L. In one vessel the glucose was uniformly labeled with C^{14} and the pyruvate was unlabeled, and in the other the glucose was unlabeled and the pyruvate was labeled with C^{14} in the carbonyl carbon. After a two-hour incubation period, the muscle was removed and treated with boiling 30% KOH; the glycogen was isolated and hydrolyzed to glucose; glucose determinations by the Nelson method were made on one aliquot and the rest was precipitated as the glucosazone, purified, recrystallized, plated and counted with a proportional flow counter. Aliquots of the incubation medium were analyzed chemically for glucose, pyruvate and lactate and an aliquot was precipitated as pyruvate dinitrophenylhydrazone, which, after purification and recrystallization, was plated and counted. The respiratory CO_2 was recovered from the center well alkali, precipitated as $BaCO_3$, plated and counted.

---

[1] This work was supported in part by a contract between Harvard University and the Atomic Energy Commission.

Between 75 and 80 per cent of the glucose and pyruvate disappearing from the medium was accounted for as lactate appearing, glycogen synthesized, CO_2 produced, protein and lipid made, and as glucose converted to pyruvate. The remainder is apparently present at the end of the incubation as other intermediates. The rate of glucose utilization is the same in the presence or absence of added pyruvate; it is increased by the *in vitro* addition of insulin and by adrenalectomy and is decreased in muscle from alloxan-diabetic rats. The hexokinase reaction is thus not affected by the presence of pyruvate in the concentration used. However, the amount of glucose carbon metabolized to CO_2 is less when pyruvate is present, the difference being greatest in muscle from adrenalectomized rats. The amount of glycogen synthesized is increased when both glucose and pyruvate is present, over that made when either is present alone. Apparently the rate at which glucose-6-phosphate is converted to glycogen is high enough to handle both the glucose-6-phosphate made from glucose by hexokinase and that made from pyruvate by the reversal of glycolysis. The addition of insulin triples the percentage of glycogen made from glucose and decreases the percentage made from pyruvate to one-third or one-fourth that in the absence of insulin. The percentage of CO_2 derived from glucose is greatly decreased in the presence of pyruvate but the percentage of CO_2 derived from pyruvate is the same or slightly increased in the presence of glucose. These results are to be expected since the metabolism of glucose to CO_2 involves pyruvate as an intermediate.

The data obtained are in agreement with results obtained previously, that insulin has a primary effect in stimulating the hexokinase reaction and an additional, lesser effect on the reactions involved in the condensation of pyruvate with oxalacetate to enter the Krebs tricarboxylic acid cycle.

The nature of the electrolyte pump in the isolated surviving frog skin. Ernst G. Huf.

Experiments were undertaken to study the influence of ATP in Ringer's solution upon the mechanism of salt accumulation at the chorion side of surviving frog skin, with Ringer's solution of 0.4 its physiological concentration on both sides. From the results obtained, it seems that the salt pump in frog skin does not make use of the energy liberated during dephosphorylation of added ATP, although it is known from other experiments that active salt uptake by the skin is closely tied up with certain metabolic reactions. It was noticed, however, that the net accumulation of NaCl at the chorion side of the skin was diminished by ATP-sodium as compared to respective controls. Inhibition of net salt accumulation could be seen also when Ringer's solution was supplemented by either sodium-glycerophosphate or sodium-phosphate or additional NaCl. The Na^+ levels, which the skin had established at the chorion side after several hours, were nearly the same for experiments with supplemented Ringer's and for their respective controls, even when the experiments started off with a 40% higher Na concentration at the chorion side as compared to the controls. The Cl^- levels at the chorion side were the same or lower than the Na^+ levels in the experiments with NaCl and phosphates as supplements, respectively. In the latter case, phosphate replaced chloride stoechiometrically. Water moved across the skin in the direction of active salt uptake. The fluid shift was greater in the experiments with supplemented Ringer's than in the controls, except in the experiments with ATP, where the opposite relationship was found. The results seem to indicate that the skin at its inside (chorion layer) tries, first of all, to establish a constant Na^+ level. The Cl^- level may vary, depending on the presence of other anions, such as phosphate. This suggests that the salt pump in isolated frog skin is a cation (Na^+) rather than an anion (Cl^-) pump, whose activity is regulated by the Na^+ concentration at the chorion layer of the skin.

Probable structural changes in neural excitation and the role of certain ions. JULIAN A. TOBIAS. No abstract submitted.

Experiments supporting the phase-boundary theory of bio-electricity. T. CUNLIFFE BARNES AND R. BEUTNER.

The recent important researches of Lorente de Nó (*J. C. C. P.*, 1949, **33**) confirmed our observation that tetracovalent onium compounds have extraordinary electrogenic action (*Anat. Rec.*, 1947, **99**: 618). Nerves of lobster or frog are placed in isotonic glucose. The electrolyte-free nerve is suspended between two watch glasses of glucose connected to a Speedomax Recording Potentiometer. Typical spikes are produced by adding an electrolyte first to one then to the other watch glass. The salt-free nerve is sensitized almost equally to common electrolytes such as NaCl, KCl, $CaCl_2$, $MgCl_2$, tetraethylammonium bromide and acetylcholine chloride. Considerable variation in the spike height was found but tetraethyl onium usually gave larger spikes than acetylcholine. A thread soaked in guaiacol gave smaller spikes with all electrolytes than did nerve. Frog sciatic was treated with an adrenergic oil (triacetin) which enhanced the potential produced by benzedrine.

Conduction in nerve is imitated in a glass tube containing an oil-saline interface. Recording from spaced outlets along the "nerve" tube showed that decrement of the negative wave was pronounced. The velocity is too great for ordinary recording instruments to measure. The wave is not produced by spreading of acetylcholine along the surface.

Sensitization can be demonstrated by treating the oil surface with a positive substance. In rabbit gut, the pilocarpine contraction is potentiated one-third by 3 mg. % sodium benzoate. In the oil-cell, acetylcholine has very little effect after atropine but duponal is able to partially restore the acetylcholine effect after atropine.

Complex formation in protein solutions obtained by mild extraction of skeletal muscle. WILLIAM R. AMBERSON, R. DALE SMITH, SYLVIA HIMMELFARB, CAROLYN STOUT AND HANS HOCH.

By the method of "mild extraction" proteins are extracted from whole muscles of exsanguinated rabbits (*J. Biol. Chem.*, 1949, **181**: 405). In recent studies the extracting fluid has been 10% potassium pyrophosphate solution, initially at or near $pH = 10.0$, but drifting to lower pH values, between 8.3 and 9.6, as extraction proceeded. This solution extracts a group of proteins, including both myogen and myosin. By the usual tests neither actin nor actomyosin has been detected in the extracts. Extraction continues for many weeks, with new solution applied periodically. At the end of three months total protein extracted amounts to approximately nine per cent of the wet weight of the muscle. The muscle imbibes fluid during extraction, but, in spite of the loss of sixty per cent of its original protein, its basic form and structure are maintained. The extracts of white muscle are clear and nearly colorless; in red muscle extracts myoglobin appears.

Electrophoretic analyses are made after dialysis against potassium phosphate buffers, usually 0.1 or 0.2 M, at $pH = 7.6$. When total protein is low the electrophoretic patterns disclose four components, A, B, C (myosin) and D, in order of increasing mobilities. At higher protein concentrations a fifth component C' appears with mobility intermediate between C and D. When it is present the patterns are asymmetrical, with C' much larger on the descending than on the ascending limb. When total protein concentration rises above 3 grams per cent, C' may include sixty per cent or more of the area of the pattern. At the same time the percentage of myosin falls and the form of the D elevation is modified until it appears to fuse with the base of C'. Such extracts are viscous and show a strong positive flow birefringence.

Such observations lead to the conclusion that C' is a protein complex, probably of high molecular weight, formed by the union of C (myosin) and D. Present evidence suggests that it is not actomyosin since (1) its viscosity is higher than that of actomyosin, (2) ATP does not reduce the viscosity as it does that of actomyosin, and (3) it dissociates over a range of salt and protein concentrations where actin and myosin combine, or precipitate, as actomyosin. The separated D fractions give a positive Biuret test and show an absorption maximum at 260 mμ, or slightly below, a value characteristic for nucleic acid. The whole extracts show an absorption maximum at 279 mμ. It is suggested that C′ may tentatively be called "nucleomyosin."

Histological studies show that pyrophosphate extraction quickly removes the normal positive birefringence of the muscle fibers, without destroying such fine details of structure as Z lines and myofibrils. If protein diffusion space is restricted during extraction by placing the muscles in cellophane bags, birefringence persists for a longer time.

The observations are consistent with the view that normal birefringence is largely caused by the presence in the fibers of nucleomyosin, rather than by actomyosin. The hypothesis is advanced that myosin may shift from one fibrous protein system to the other, during contraction and relaxation. Extreme changes in muscle viscosity may thus be avoided. Nucleomyosin may be tentatively considered as a native protein of the living muscle cell.

Some actions of ryanodine on muscle. ROBERT RAMSEY, MARY LOUISE FLINKER AND LESLIE EDWARDS. No abstract submitted.

Experiments on spiracles. JOHN B. BUCK AND MARGARET L. KEISTER.

In mature Phormia larvae 11% of the oxygen normally taken up from air crosses the cuticle, the rest entering via the one pair each of anterior and posterior spiracles. Uptake is identical in normal larvae in air or oxygen and in larvae with anterior spiracles ligated. With posterior spiracles alone ligated, uptake is 55% of normal in air, but normal in oxygen. Further evidence that the capacity of the anterior spiracles is much lower than that of the posterior is (1) larvae with only the anterior spiracles ligated darken evenly and rapidly at pupation whereas those with only the posteriors ligated darken slowly and unevenly from the front; (2) larvae in osmic vapor blacken first posteriorly.

In larvae DDT-poisoned before ligating the anterior spiracles, the posterior spiracles pass twice the oxygen used normally, but not enough for the stimulated metabolism unless the pO_2 is raised.

The posterior spiracles are not "without closing apparatus" (Imms). Each is covered by a delicate membrane having arborizations of flat transparent fibrils and three slits overlying the traditional "spiracular openings." The slits open reversibly when perfused with glycerin.

The total area of the three slits, fully open, is only 1/60 the cross-sectional area of the tracheae leaving the posterior spiracle. If gas transport is by diffusion alone (Krogh) the disproportionate smallness of the spiracular openings makes better physical sense if the volume passed is, as in leaf stomata (Brown and Escombe), proportional to a linear dimension of the apertures, rather than to their area. Supporting this hypothesis, (1) the total slit perimeter is 2/3 the total posterior tracheal perimeter; (2) the ratio of the tracheal perimeter at the anterior spiracle to that at the posterior equals the ratio of the maximum possible oxygen uptakes from air through the respective spiracles.

A new technique for the study of biological structures in the electron microscope. THOMAS F. ANDERSON.[1]

In the conventional air drying of biological specimens for the electron microscope the surface tension of the evaporating liquid flattens all but the most rugged specimens. Such artifacts are minimized in the following method of drying in which ideally *no* phase boundary moves through the specimen. After being fixed, the specimen in water on a formvar film is dehydrated by passing it through a series of water-alcohol mixtures and eventually into amyl acetate. The specimen is then placed in a pressure bomb and the amyl acetate is flushed out with liquid carbon dioxide below its critical temperature, 32°. Then the bomb, completely filled with pure liquid carbon dioxide is warmed to a temperature *above* 32° where the liquid

---

[1] These studies were aided by a contract between the Office of Naval Research and the University of Pennsylvania (NR 135–197).

changes imperceptably to a gas and its surface tension vanishes. The gas is then allowed to escape from the bomb and the dry specimen is ready to be placed in the electron microscope for study.

Stereoscopic electron micrographs of such delicate specimens as bacteria, hemolysed human erythrocytes, striated muscle, protein gels, and the cilia and trichocysts of *Paramecium caudatum* show structures which do not collapse when dried by this method. A number of new morphological features have appeared, each of which merits further detailed study.

The role of phosphagen in fertilization of the sea urchin egg. EDWARD L. CHAMBERS AND THOMAS MENDE.[1]

The unfertilized eggs of *Asterias forbesi* and *Strongylocentrotus drobachiensis* were homogenized in ice cold 5 per cent trichloracetic acid, the homogenate centrifuged, and the precipitate discarded. Barium and ethanol fractionations were carried out on the extract according to the method of Le Page and Umbreit. On the alcohol insoluble fraction inorganic phosphate was determined before and after hydrolysis for one minute in 2 per cent trichloracetic acid at 100°C., and arginine was determined by the method of Sakaguchi-Weber. The quantity of arginine determined agreed closely with the quantity calculated from the inorganic phosphate liberated in one minute at 100°C. in 2 per cent trichloracetic acid, indicating the presence of arginine phosphate, in the amount of 570 μg. arginine phosphate per ml. of *S. drobachiensis* eggs and 270 μg. per ml. of *A. forbesi* eggs. Creatine phosphate could not be demonstrated.

The quantity of inorganic phosphate before and after one minute hydrolysis at 100°C. in 2 per cent trichloracetic acid (1 minute labile phosphate) and after seven minutes hydrolysis at 100°C. in 1 *N* HCl (7 minutes labile phosphate), and the quantity of total phosphate was determined on the trichloracetic acid extracts of unfertilized eggs of *S. drobachiensis* and of fertilized eggs at various intervals after insemination up to the time of first cleavage. Within the first six minutes after fertilization a prominent decrease of inorganic phosphate and a marked increase of one minute labile phosphate (arginine phosphate) were demonstrated. Further alterations in the same direction with minor variations were observed up to the time of first cleavage. The seven minute labile phosphate and the total phosphate values remained constant. The incorporation of inorganic phosphate in arginine phosphate and acid stable phosphorus containing compounds accounts entirely for the decrease of inorganic phosphate following fertilization.

Effects of nitrate on muscle and nerve responses. ARTHUR J. KAHN AND ALEXANDER SANDOW.

Isometric mechanical responses of frog sartorii stimulated by slightly supermaximal massive, transverse, twitch shocks have been recorded by the piezoelectric, cathode-ray oscillographic method for the latency events and by optical myography for peak developed tension. The normal medium was a phosphate buffered Ringer's solution, and the experimental, a similarly buffered nitrate-Ringer's (all the usual chloride replaced by nitrate). Within about 10 min. after submersion of a muscle in the nitrate-Ringer's, its behavior relative to that just previously obtained in chloride-Ringer's shows the following changes: (1) latent period events are slightly hastened, (2) the depth of the latency relaxation is increased by about 30%, and (3) the developed peak tension is increased 2 to 3 fold. The altered behavior is maintained in the nitrate-Ringer's for several hours and upon replacement of the muscle in chloride-Ringer's normal responses are restored in about 10 minutes.

The potentiated tension output in the nitrate-Ringer's is not due to recruitment of fibers, since the shocks throughout were supermaximal; nor is it due to tetanization, since the single applied shock leads to only a single action potential. It is therefore concluded that the augmented tension output in the nitrate-Ringer's results from an alteration of the fundamental mechanism of contraction of each fiber. But, since the nitrate effects are so quickly produced and reversed, it is inferred that the direct action of this agent is on the excitatory membrane of the fibers and that the potentiated mechanical response results indirectly from a modification of the coupling of excitation to the contractile mechanism. The alterations in the nitrate-latency events may be evidence for the assumed changes in coupling of excitation to contraction.

---

[1] Under grant from the N. C. I., U. S. P. H. S.

In pursuance of the view that nitrate directly affects excitatory events, it has further been shown that it increases the excitability of muscle fibers and also of nerve fibers of the frog sciatic trunk, and it causes an increase in the spike of the action potential of both muscle (about 25%) and of at least the alpha fibers of the sciatic nerve (25 to 75%). The potentiation of the action potential, however, has not been regularly obtained and further work is needed to establish this effect of nitrate.

Photochemical inactivation of chymotrypsin. Harold F. Blum, Charlotte T. Pope, Judith P. Price and Ralph Witmer.

In activation of crystalline chymotrypsin by ultraviolet radiation absorbed by this protein ($\lambda = 0.2537\,\mu$, and polychromatic $< 0.32\,\mu$); and photosensitized inactivation with rose bengal ($\lambda = 0.546\,\mu$) and with riboflavin ($\lambda = 0.365\,\mu$) was compared.

Inactivation by ultraviolet goes on at the same rate in very low partial pressure of O_2 as in air. Photosensitized inactivation, on the other hand, is almost completely abolished in the absence of O_2; so nearly so that any residue of effect may be attributed to failure to remove O_2. These findings are in agreement with earlier studies serving to distinguish between the effects of ultraviolet radiation and photosensitization by dye (photodynamic action) in living organisms.

Some effects of x-rays on the mechanisms of cell division. Titus C. Evans.

It has been found in the eggs of *Arbacia punctulata* and of *Ascaris equorum* that roentgen radiation will delay division in such a way as to upset the usual chronological correlation of certain observed phenomena with the cleavage process.

Prolonged exposure of Arbacia eggs to X-radiation at low intensities allows subsequent division without further delay, upon cessation of the irradiation. This indicates a recovery rate approximately equal to the rate of inhibition. However, the longer the exposure, the more pronounced the subsequent effect on the development of the embryos. This indicates a different recovery rate for the two processes.

Irradiation of Ascaris eggs at developmental temperature allows recovery (cleavage delay) during the longer exposures. Irradiation at subdevelopmental temperatures reduces the recovery rate as regards cleavage time, but does provide recovery as regards the subsequent development of the embryos. Irradiation of eggs at developmental temperatures, with subsequent storage at 5°C. for several months, allows recovery as regards developmental injury. However, it enhances the delay in first cleavage at the longer radiation exposures.

The uptake of radioactive phosphorus in Arbacia eggs increases rapidly upon fertilization. This uptake and cleavage rate are reduced by lowering the temperature. On the other hand, irradiation will delay cleavage without affecting the rate of P^{32} uptake. Likewise, there is a sudden and rapid increase in oxygen consumption of Arbacia eggs upon fertilization which is not affected by irradiation strong enough to retard cleavage.

Direct spectroscopic observations on cytochrome oxidase and its reaction with carbon monoxide. Eric G. Ball and Octavia Cooper.

The turbid heart muscle preparation previously described (*J. Biol. Chem.*, 1949, **180**: 113) is treated with 2 per cent solutions of desoxycholate in glycylglycine buffer pH 7.4 to yield a relatively clear preparation. This preparation is reduced by the addition of dithionite, succinate, ascorbate or ferrocyanide in an evacuated Thunberg tube fused to an optical cell. The spectrum of the reduced cytochromes is measured and carbon monoxide then admitted to the cell and the measurements repeated.

Complete and rapid reduction of all the cytochromes is obtained only with dithionite; succinate reduces cytochromes a and c completely, b partially; ascorbate reduces cytochromes a and c completely, but not b; ferrocyanide reduces cytochrome c approximately 15 per cent and cytochrome a 50 per cent. In the case of succinate, ascorbate and ferrocyanide, the reduction rate is slow unless extra cytochrome c is added. Addition of CO to preparations reduced with dithionite, succinate or ascorbate causes the band centered at λ 605 mμ to diminish and produces a new band located at λ 590 mμ; a secondary band at λ 540 mμ also appears. These reductants also produce a band located at λ 443–445 mμ, which is most clearly defined with ascorbate. This band disappears on the admission of CO. This band does not appear in preparations reduced with ferrocyanide, nor does the portion of the band at λ 605 mμ produced by ferrocyanide shift with CO.

As a working hypothesis, it is proposed that the band at λ 605 mμ is composed of two substances, both of which function as cytochrome oxidase. One has a higher oxidation reduction potential than the other, and does not show any reaction with CO spectroscopically. The other of lower potential, not reduced by ferrocyanide, possesses a characteristic band at λ 443–445 mμ in addition to its absorption at λ 605 mμ, and both of these bands shift in the presence of CO.

Oxidase activity—light-absorption relationships in cytochrome system of heart muscle preparations. BRITTON CHANCE.

The sequence of chemical reactions between oxygen and succinate that occur in heart muscle preparations has been recently summarized by Slater (*Biochem. J.*, 1950, **46**: 484) and closely follows the mechanism of Keilin and Hartree (*Proc. Roy. Soc. B.*, 1939, **127**: 167). This sequence of cytochrome action has been based largely upon the effect of inhibitors (especially hydrocyanic acid and carbon monoxide) upon the absorption spectrum and upon the enzymatic activity of the heart muscle preparations. However, in no case heretofore has there been a direct correlation between the amount of the cytochrome component acting and the oxidase activity. By means of rapid methods for measuring oxidase activity and sensitive spectrophotometric methods for detecting the cytochrome components, studies have been made of the effect of a) oxygen concentration upon oxidase activity and upon the state of oxidation of the cytochrome components, and b) the effect of cyanide upon the oxidase activity and upon the amount of cytochrome component acting.

The platinum microelectrode (*Rev. Sci. Instruments,* 1942, **13**: 524) has been used to measure the rate of oxygen consumption, and spectrophotometry has been used at 250 mμ to measure the succinate $\rightleftharpoons$ fumarate rate and at 445 mμ to measure the optical density of the heart muscle preparation corresponding to the oxidation and reduction of Keilin and Hartree's a_3 component. The correlation of the decrease of oxygen and fumarate rates with the decrease in the concentration of oxidized a_3 component as the oxygen concentration falls to zero suggests that the concentration of the oxidized a_3 component determines the activity of the preparation. Similar conclusions are reached when a portion of the oxidized a_3 component is bound by cyanide. Preliminary numerical values are: oxygen affinity $\leqslant 1 \times 10^{-6}$ M, cyanide dissociation constant $\sim 1 \times 10^{-5}$ M, based upon both spectrophotometric and activity data.

Oxidation and reductions catalyzed by isolated chloroplasts. ALAN H. MEHLER AND HANS GAFFRON.

Isolated chloroplasts have been shown by many investigators to carry out model reactions of two components of normal photosynthesis: reduction using electrons from water, and, simultaneously, liberation of molecular oxygen. In spite of certain differences between the abilities of intact cells and isolated chloroplasts to reduce various compounds, the present studies on the mechanisms used by isolated chloroplasts are considered an approach to the understanding of the light reaction of photosynthesis.

The possibility that hydrogen peroxide might be an intermediate in the evolution of oxygen from water was tested by carrying out Hill reactions with spinach chloroplasts in the presence of catalase and ethanol, which use hydrogen peroxide very efficiently at low concentrations to form acetaldehyde. No hydrogen peroxide was detected during the course of Hill reactions. However, in the presence of air and the absence of Hill reagents, acetaldehyde was found, showing hydrogen peroxide formation by illuminated chloroplasts. It is concluded that hydrogen peroxide is not an intermediate in the liberation of oxygen, but can be formed by reduction of molecular oxygen serving as a Hill reagent.

The rate of oxygen consumption by chloroplasts with catalase and ethanol is significantly slower than oxygen evolution with quinone at equal illumination. When quinone, catalase and ethanol are added to chloroplasts and illuminated, oxygen is first evolved at the rate characteristic of quinone, then, after about 90% of the theoretical evolution, oxygen consumption begins, and maintains a rate two to three times that of the control without quinone. The relations obtained with these systems have allowed this tentative hypothesis to be advanced: that a first reaction of electron transfer can be inhibited by a naturally occurring substance and the inhibition reversed by quinone, and that narcotics inhibit a second enzymatic electron transfer.

The influence of glycolytic factors on the potassium and sodium content of the yeast cell. GEORGE T. SCOTT AND MARC JACOBSON.[1]

The carbohydrate metabolism of the cell has been shown by a number of investigators to be associated with the accumulation and retention of potassium.

Experiments were carried out on 50 ml. samples of 2% suspensions of Fleishmann's baker's yeast in sodium enriched Williams solution placed in Goetz centrifuge tubes and maintained under anaerobic conditions. Total cell volumes were determined after centrifuging. The cells were ashed and analyzed by flame-photometry.

Time-course curves were obtained relating the loss of potassium from the cells at varying concentrations of the glycolytic inhibitors iodoacetic acid and sodium fluoride. The loss of this element in the presence of the inhibitors does not parallel the corresponding inhibition of fermentation. The data demonstrates a high sodium permeability.

In the absence of substrate in the medium approximately 30% of the maximal potassium content is lost. The remaining 70% is retained at a constant level for at least 180 hours. In the meantime the hydrolyzable polysaccharide content of the cell is declining. It is assumed the potassium retention is maintained at the expense of glycolysis.

Pyruvate given prior to sodium fluoride reduces the loss of potassium from the cell. The observation suggests the formation of a protective unit by combination of the enzyme with a compound formed from added pyruvate, or pyruvate is rapidly generating a compound which results in the retention of a significant proportion of the cellular potassium.

Anomalous penetration of phosphate into the gills of Mytilus. R. R. RONKIN.

Pieces of excised ctenidium from a single demibranch of *Mytilus edulis* L. were placed in a special glass irrigation chamber (*J. C. C. P.*, 1950, **35**: 241) in a continuous flow of artificial sea water (ASW) containing radioactive phosphorus (P^{32}) as phosphate, but no additional phosphate except for that occurring as impurities in the other components of the ASW. Such solutions contained 5.3×10^{-6} mols$\cdot$l^{-1} of phosphate-P^{31} and about 1×10^{-11} mols$\cdot$l^{-1} of phosphate-P^{32}. Two other solutions contained the same concentration of phosphate-P^{32}, plus added "carrier" phosphate-P^{31} to bring the total phosphate concentrations to about 100×10^{-6} and 500×10^{-6} mols$\cdot$l^{-1}, respectively. Penetration of phosphate into the ctenidium from these three solutions was studied as a function of time and total external phosphate concentration, by means of a Geiger-Müller counting tube placed just above the thin glass cover of the chamber. The rate of penetration of phosphate from the ASW which contained the highest phosphate concentration (500×10^{-6} mols$\cdot$l^{-1}) was two to three times higher, compared with rates of penetration from the other solutions, than could be accounted for on the basis of a direct linear relationship between penetration rate and external phosphate concentration. The order in which the solutions were administered to a given gill fragment did not affect this disproportionality. When irrigant solutions were changed, the new penetration rate was observed to be in effect as soon as a reading could be made, and reversion to the previous rate became evident immediately upon switching back to the original irrigant. The apparently anomalous penetration from the ASW with the highest phosphate concentration may be associated with the presence of particulate (colloidal) $CaHPO_4$ in the ASW. The possibilities of a greater rate of penetration owing to direct entrance of the particles into the cells, or by an increase in solubility of the salt in the immediate environment of the cell surface, were discussed.

---

[1] Aided by a contract with the Office of Naval Research with Oberlin College.

Effect of stretch on conduction in single nerve fibers. THEODORE HOLMES BULLOCK, MELVIN J. COHEN AND DYREL FAULSTICK.[1]

Species whose nerve fibers tolerate considerable stretch offer an opportunity to study the effect of change in physical parameters of the membrane and current path upon function. Measurements of electrical dimensions have not yet been made but should be of interest in view of preliminary results.

The median giant fiber of *Lumbricus terrestris* has been so prepared that simultaneous measurements could be made of diameter and rate of conduction under various degrees of stretch. Stimulus-response time measurements have been checked by frequent use of two-channels of pickup, proximal and distal to stimulating electrodes. These fibers will permit reversible series of estimations over a two to one range of fiber diameter. In order to make this figure accurate it was necessary to measure exactly the same point on the fiber before and after stretching, but cruder measurements without this precaution also show that diameter is actually decreasing rather than stretch merely straightening out a sinuous fiber. The result in almost every case was no change in conduction rate (per meter).

Experience in recognizing the degree of stretch necessary to assure just complete straightening permitted use of data from preparations in which diameter could not be measured. Intact, unanesthetized worms from which action potentials were recorded during spontaneous movements upon simple mechanical stimulation give exactly the same result as strip preparations of the midventral body wall or preparations of the isolated cord. One can simply take the maximum rate attained under various degrees of stretch or of extension of the intact animal as the correct figure for the straightened nerve fiber.

Three specimens, one in each type of preparation, have given anomalous results (out of more than twenty-five cases), namely, clear increases in rate. Bullock (*J. Neurophysiol.*, 1945, **8**: 55) reported increase in rate in similar experiments without diameter control. Re-examination of the scatter of points on the original graphs shows that all cases except one—that having the most points and therefore chosen for publication—permit the interpretation that two slopes intersect, the one lower on the length axis corresponding to expectation from straightening out a sinuous fiber, that higher on the length axis to constant rate. In view of the overwhelming majority of cases giving constant rate, the conclusions of that date must be amended. Thus the conclusions of Jenkins and Carlson (*J. Comp. Neurol.*, 1904, **14**: 85) and Carlson (*Amer. J. Physiol.*, 1905, **13**: 351) are confirmed, although their interpretation, that this is "evidence of the fluidity of the conducting substance," seems less satisfactory today.

We have found the same relation in the median and lateral giants in *Lumbricus*, in the third order giants of *Loligo pealii*, and in the central giants of the polychaetes *Lumbrineris erecta*, *Marphysa sanguinea* and *Eudistylia polymorpha*. Tolerance of stretch varies greatly, the squid permitting very little and the median giant of the earthworm much less than the lateral, for example.

Since core dimensions and presumably conductance can decrease greatly without depressing rate, contrary to generally accepted rules, it is to be hoped that impedance measurements will be made with a view to revealing possible submicroscopic folds and the mechanism of exact compensation of conduction rate.

---

[1] Grants in aid from the University Research Committee, the Rockefeller Foundation and the Public Health Service are gratefully acknowledged.

ABSTRACTS OF SEMINAR PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY

July 5, 1950

Alkaline phosphatase activity in normal and scorbutic bones. Anita Zorzoli.

The deficiency disease, scurvy, is characterized in part by a disturbance in the formation and calcification of the organic matrix of the bones of the body. It has been suggested that the inhibition of calcification occurs as a result of the inhibition of the enzyme, alkaline phosphatase.

In this study young adult guinea pigs were maintained on a scorbutogenic diet for 26 days and then sacrificed for study. The proximal end of one tibia was fixed, decalcified, embedded in paraffin and sectioned for study of the histochemical localization of alkaline phosphatase. The other tibia was weighed, homogenized in distilled water and allowed to autolyze at room temperature for two days. A suitable aliquot was used for the chemical estimation of phosphatase activity.

Both chemical and histochemical methods demonstrated a significant decrease in phosphatase activity of the scorbutic bones as compared with the normal controls. Expressed as micrograms of phosphorus liberated per gram of tissue per hour of incubation, the experimental animals had an average value of 4.16 and the normal controls of 8.04. The decrease in enzyme activity was widespread throughout the bone. This was especially true of the region of articular cartilage bordering on the secondary zone of ossification and in the columnar and hypertrophic cartilage regions, where the intracellular and extracellular phosphatase activity is normally very high. As indicated by the degree of blackening, the altered osteoblasts of the scorbutic animals showed little or no phosphatase activity.

One group of scorbutic animals was given a single, large dose of vitamin C and then was sacrificed at 12, 24 and 48 hours after the treatment. At 12 and 24 hours the phosphatase activity was the same as in the untreated scorbutics. At 48 hours the activity had risen to a value in the order of 30% above the normal controls.

Reduction of Janus Green by isolated enzyme systems. S. J. Cooperstein and A. Lazarow.

Since the supravital staining of mitochondria by Janus Green is dependent upon oxygen tension and is reversibly abolished by cyanide, (Lewis and Lewis in Cowdry's "General Cytology," 1924) a study of the reactions of Janus Green with isolated enzyme systems was undertaken. Upon reduction, the blue Janus Green is converted successively to leuco-Janus Green (reversibly), to red safranine (irreversibly) and to colorless leuco-safranine (reversibly). These changes can be followed by concomitant changes in the absorption spectra.

Components of the following respiratory chain were studied for their reactions with Janus Green and its reduction products:

$$\text{Substrate} \xrightarrow{\text{Specific Dehydrogenase}} \text{Diphosphopyridinenucleotide (DPN)} \rightarrow \text{Flavoprotein} \rightarrow \text{Cytochrome } c \rightarrow \text{Cytochrome oxidase} \rightarrow \text{Oxygen}$$

Under anaerobic conditions substrate amounts of DPN-H_2 (reduced diphosphopyridinenucleotide) reduced Janus Green only in the presence of catalytic amounts of flavoprotein (Straub's). Therefore, FLAV-H_2 (reduced flavoprotein) is the component which reduces Janus Green. Likewise dehydrogenase systems capable of reducing DPN also reduce Janus Green in the presence of catalytic amounts of flavoprotein. With the lactic dehydrogenase system the reduction of Janus Green is so rapid that it may be accomplished aerobically; with the glucose dehydrogenase system however, anaerobic conditions must be used because in the presence of oxygen the leuco-Janus Green formed is immediately reoxidized to Janus Green and visible reduction is not apparent.

Using the lactic dehydrogenase system under aerobic conditions, the addition of cytochrome *c* plus a cytochrome oxidase preparation prevented the reduction of Janus Green. Evidence has been presented suggesting that one of the reduction products of Janus Green, *i.e.*, leuco-Janus Green, reduced cytochrome *c* and thereby became reoxidized to Janus Green.

These results, therefore, indicate that whereas Janus Green can be reduced by FLAV-H_2 or any dehydrogenase-DPN-flavoprotein system, this reduction can be prevented by the cytochrome *c*-cytochrome oxidase enzyme system. The addition of these latter components would tend to keep the flavoprotein in its oxidized state, and at the same time it would reoxidize the leuco-Janus Green formed.

The reduction of Janus Green by liver cell constituents and a proposed mechanism for the supravital staining of mitochondria. A. LAZAROW AND S. J. COOPERSTEIN.

Mitochondria prepared from rat liver by either the saline or hypertonic sucrose methods reduced Janus Green anaerobically in the presence of sodium lactate. The addition of DPN or nicotinamide facilitated the reduction. The supernatant following the removal of mitochondria, or that following the removal of mitochondria plus the submicroscopic particulate components, likewise reduced Janus Green; lactate, DPN, and nicotinamide facilitated the reaction.

Janus Green forms non specific insoluble complexes with amino acids and proteins (gelatin and soluble liver proteins). Mitochondria prepared in either 0.85% saline, 0.88M sucrose, or in the latter followed by washing with saline, were equally effective in adsorbing Janus Green from a 1:10,000–1:20,000 solution, leaving little unadsorbed dye in solution. On microscopic examination of these preparations the mitochondria suspended in saline were markedly swollen granules which stained faintly blue, whereas those suspended in sucrose were deeply stained blue rods. The apparent failure of mitochondria suspended in saline to stain with Janus Green is attributed to their swelling rather than to a lack of adsorption of the dye. Likewise microscopic staining with Janus Green is not positive proof that isolated granules are mitochondria.

It has long been known that mitochondria become decolorized when oxygen is removed or cyanide is added. Lewis and Lewis (1924) found that when tissue cultures were supravitally stained with Janus Green the mitochondria became decolorized upon the addition of cyanide. Removal of the cyanide, without further addition of dye, restored the stain. Since cyanide is a known inhibitor of cytochrome oxidase, it would appear that this enzyme plays a role in the supravital staining of mitochondria.

Therefore the supravital staining of mitochondria within the intact cell appears to be dependent upon: (1) the non-specific adsorption of Janus Green on protein, (2) the rapid reduction of Janus Green in the non-mitochondrial portions of the cell, and (3) the localization within the mitochondria of the cytochrome system which slows or prevents the reduction of Janus Green at this site.

JULY 11, 1950

The enzymatic conversion of ovalbumin to plakalbumin. C. A. VILLEE, K. LINDERSTRØM-LANG, M. OTTESEN, C. B. ANFINSEN AND D. STEINBERG.

Bacillus subtilis produces a proteinase which acts on ovalbumin to split off 1.2% of its nitrogen as peptides and transforms the remainder into a second, crystallizable protein, plakalbumin. By starch column chromatography and photometric ninhydrin analyses of the effluents these peptides were separated and their constituent amino acids determined. The peptides were obtained by precipitating the protein, after a given reaction period, with 10% trichloracetic acid and extracting the TCA with ether. The aqueous solutions of peptides were lyophilized and aliquots were chromatographed with and without hydrolysis. For the first five hours of reaction, the peptides on hydrolysis yielded only valine, alanine, aspartic acid and glycine. Chromatography of unhydrolyzed peptides revealed three fractions, A, B and C, no matter what solvent mixture was used. The appearance of fractions A and C in the reaction is correlated with the ammonium sulfate titration, shown by Linderstrøm-Lang and Ottesen to be a measure of the conversion of ovalbumin to plakalbumin. In contrast, fraction B is produced by a second reaction, not correlated with this conversion.

The peptides isolated from one column were hydrolyzed and chromatographed on fresh columns. Fraction A proved to be a hexapeptide, 1 valine: 3 alanine: 1 aspartic acid: 1 glycine; fraction B, a tetrapeptide, containing the four amino acids in equimolecular amounts; and fraction C, the dipeptide alanyl alanine.

The terminal amino acid of all three peptides was shown to be alanine by the Leonis-Levy method involving the reaction with CS_2 in alkaline solution to give a dithiocarbamic acid and the subsequent ring closure in acid solution to give a thiazalone. This is extracted with ether, hydrolyzed to give the free amino acid, and the latter identified by filter paper chromatography.

Radioactive egg albumin, prepared by incubating minced hen's oviduct in buffered salt solution containing $NaHC^{14}O_3$, and precipitated by $(NH_4)_2SO_4$ was diluted with carrier and recrystallized three times. This was used as substrate in the enzyme reaction and the peptide and plakalbumin fractions were separated as before. Aspartic acid was isolated by filter paper chromatography from the hydrolysate of each and its specific activity determined. The peptide aspartic in each of four experiments had approximately twice the specific activity of the plakalbumin aspartic. This suggests that in the synthesis of ovalbumin, the constituent amino acids do not simply fall into place on a waiting model from a uniform amino acid pool, but that the aspartic of the hexapeptide, and perhaps the whole hexapeptide portion, enters the molecule differently, either from a different peptide pool or via steps with a different rate.

The denaturation of ricin as affected by pH and temperature. MILTON LEVY AND ANGELO E. BENAGLIA.

The effects of pH and temperature on the rate at which crystalline ricin becomes insoluble in a solution adjusted to its isoelectric point can be rationalized on the following postulates: (a) A number of ionic forms of the protein related through mass action equations involving hydrogen ion exists. (b) Each of these ionic forms is characterized by a specific first order rate constant with respect to the formation of an insoluble material. (c) The theory of absolute reaction rates is applicable for the description of temperature effects on the rate constants. Summations of the individual rates with respect to the amounts of each of the ionic forms as related by the dissociation constants and pH gives the total rate for a given pH and temperature. Only a few (6) of the known acid-base groups (about 50) of ricin are of significance for the rate between pH 1 and 13 and these do not appear to be the usual acid-base groups of proteins. It is postulated that prototropic hydrogen bonds are the basis of the effects of pH upon the rate of denaturation of proteins.

The rationalization allows the description of the 1200 individual observations over a pH range of 12 units and a temperature range of 43° with fifteen arbitrary parameters. This material will appear in full in the *Journal of Biological Chemistry* in the near future.

The preparation and properties of the conalbumin of egg white. ROBERT C. WARNER AND IONE WEBER.

The presence of two protein components in the albumin fraction of egg white was first noted by Osborne and Campbell (*J. Am. Chem. Soc.*, 1900, **22**: 422) on the basis of thermal coagulation points. Longsworth, Cannan and MacInnes (*J. Am. Chem. Soc.*, 1940, **62**: 2580) found that this protein undergoes a reversible change when it is exposed to pHs below 4, resulting in a loss of solubility and change in electrophoretic mobility. Conalbumin also combines reversibly with ferric iron in a stoichiometric complex containing two atoms of iron per molecule of protein (*Arch. Biochem.*, 1946, **11**: 9). In order to study these properties in detail the preparation of purified conalbumin has been investigated. We have obtained both the iron complex and the iron free protein in crystalline form by a salting-out and alcohol fractionation method. These preparations are electrophoretically homogeneous except at low ionic strength at the isoelectric point. The iron complex has a more negative mobility at all pHs than the iron free conalbumin.

JULY 18, 1950

Amino acids and peptides as adjuvants in agglutination of starfish sperm by fertilizin. CHARLES B. METZ. No abstract submitted.

Extension of the functional life span of spermatozoa by amino acids and peptides. ALBERT TYLER.

In the course of investigation of the amino acids and sugars, of which the fertilizin molecule of sea-urchins is composed, the action of these substances on the sperm was tested. It was found that the addition of various amino acids and peptides extended very greatly the functional life span of the sperm. The amino acids and peptides thus far tested include glycine, dl-alanine, dl-valine, dl-leucine, l-lysine, dl-glutamic acid, dl-phenylalanine, l-tryptophane, l-cysteine, l-cystine, glycylglycine, and glutathione. All of these proved effective. The prolongation of functional life span amounted to greater than 50 fold in certain experiments with dilute (ca. 0.4%) sperm in 0.05 M glycine-sea water. Concentrations of glycine above and below this value were somewhat less effective. Optimum concentrations for the other amino acids have not as yet been determined, but preliminary results indicate that sulphur-containing amino acids are most effective in low concentration.

The animals used in this work include *Strongylocentrotus purpuratus* and *Lytechinus pictus* on the west coast and *Arbacia punctulata, Echinarachnius parma* and *Chaetopterus pergamentaceus* at Woods Hole.

The presence of the amino acids has little effect on the titer of agglutination of the sperm by fertilizin. However, the spontaneous reversal of the agglutination occurs much more rapidly. Also determinations of the liberation of antifertilizin by the sperm upon aging show that this is greatly retarded by the presence of glycine. Evidently the presence of the amino acid opposes the dissolution of this surface constituent of the spermatozoon.

Tests of the possibility that the amino acids act as nutrients showed no significant production of ammonia or of the keto-acid that would result from their oxidation. The possibility of metabolic utilization of the amino acid was also examined by determination of glycine (method of Alexander *et al., J. B. C.*, 1945, **160**: 51), after various times of incubation with sperm, and no significant change was found. It appears then that the amino acids do not serve as nutrients in prolonging the life of the sperm. Also, contrary to the prevalent view, sperm do not normally die because of exhaustion of endogenous food reserves as a result of their activity. The amino acids, in extending the life span of the sperm, maintain them in a state of high motility and high respiratory activity.

Another interesting feature of the results is that fertilization with aged sperm generally results in poor, or no, membrane elevation. In prolonging the life span of sperm, the amino acids correspondingly extend the period during which a good fertilization reaction is obtained. Evidently fertilization does not simply involve a trigger mechanism in which the spermatozoon acts in all or none fashion to pull or fail to pull the trigger. The type of response given by the egg depends not only on its own condition but on that of the spermatozoon as well.

Respiration and fertilizing capacity of sea urchin sperm in presence of serum albumin and jelly coat solution. ERIK VASSEUR, ELSA WICKLUND AND JOHN RUNNSTRÖM.

Addition of glycine or other amino acids or of serum albumin (cryst.) considerably increases the percentage of fertilization in refractive eggs of *Strongylocentrous droebachiensis, Echinocardium cordatum* or *Psammechinus miliaris.* One of the effects is the swelling of the jelly coat. The agents have, however, also proved to increase the O_2-uptake of the spermatozoa and to prolong their fertilizing life. The fertilizing capacity of a suspension of spermatozoa in sea water decreased to 50 per cent in about 100 minutes. In a similar suspension of spermatozoa in 0.2 per cent serum albumin the same decline was observed only after 14 hours. After removal of the jelly coat a higher concentration of spermatozoa is necessary to bring about 100 per cent fertilization than in eggs with jelly coat. In presence of 0.2 per cent serum albumin in the sea water, however, 100 per cent fertilization is brought about by the same sperm concentration as that used when the eggs are surrounded by a jelly coat. In confirmation of work by Gray (1928) it was found that addition of jelly coat substance to a sperm suspension causes an increase of O_2-uptake of the spermatozoa. Whereas upon addition of albumin the rate of O_2-uptake remains increased for several hours the rate declines in presence of jelly solution to the control value within about 100 minutes (*Arkiv f. Kemi,* 1949, **1**: 393). The period of fertilizing capacity of the spermatozoa is also prolonged in presence of jelly coat solution. In one experiment with *Psammechinus miliaris* spermatozoa suspended for seven hours in 0.02 per cent jelly solution gave 100 per cent fertilization, whereas the control spermatozoa in sea water gave only 5 per cent fertilization after the same time. The activating effect of the jelly substance upon the O_2-uptake of the spermatozoa can be brought about by preparations, which are hydrolysed to some extent. Conversely the sperm agglutinating property is lost even after mild hydrolysis of the jelly.

JULY 25, 1950

The effect of metabolic poisons on potassium loss from rabbit red cells. J. W. GREEN AND A. K. PARPART.

Freshly withdrawn rabbit red cells were washed in isotonic NaCl solutions which were buffered with 0.03 *M* phosphate. Washing was continued until the content of reducing substance in the cell suspensions was 15% of that in whole blood. Subsequently, this 15% decreased at the same rate in the presence or absence of inhibitor. Paired aliquots of these cell suspensions, to one of which glucose in the amount of 400 mg.% was added, were exposed to the action of 0.05 *M* sodium fluoride (NaF), 0.0005 *M* sodium azide (NaN_3), 0.0003 *M* iodoacetic acid (IAA) and 0.0001 *M* p-chlormercúricbenzoic acid (PCMB) under sterile conditions at pH 7.0 and 36.8°C. for times up to 48 hours. PCMB was very lytic and only one determination of K loss was made. The following table shows the times in hours to 40% loss of initial K value:

Poison	Cells with glucose	Cells without glucose
Controls	34	25
PCMB	0.3	0.3
NaF	13	14
IAA	16	17
NaN_3	26	19

The relatively slower loss of K in the control with glucose over that without glucose indicated that glucose added to the environment was a factor in K retention in red cells at pH 7.0. However, from the table it is clear that NaF, IAA and PCMB produced about the same rate of K loss in the presence as in the absence of glucose. This result is interpreted to mean that in these experiments the chief action of these metabolic poisons was an alteration of the cell surface rendering it more permeable to the loss of K. NaN_3 did not have this effect to the same degree.

The effect of x-rays on permeability to water and to glycols of Arbacia eggs. B. LUCKÉ, R. RICCA AND A. K. PARPART. No abstract submitted.

The permeability of erythrocytes at different temperatures. M. H. JACOBS AND H. N. GLASSMAN.

It is a common belief that a high Q_{10} temperature coefficient for the entrance of a solute into a cell indicates that a chemical mechanism of some sort is involved in the process. On the other hand, Danielli and Davson have given plausible reasons for the view that such temperature coefficients might have a purely physical origin. The present observations on the permeability of the erythrocytes of several dozen species, representing all the large classes of vertebrates, to 4 solutes (ethylene glycol, glycerol, urea and thiourea) at temperatures of 0, 10, 20, 30 and 40°C. support the physical rather than the chemical theory. In general, there is nothing characteristic about the temperature coefficient for a given solute. Urea and glycerol, in particular, show a wide and continuous range of Q_{10} values from group to group and from species to species. These values, however, in nearly all cases are closely correlated with the difficulty of entrance of the solute into the erythrocytes in question.

In making simultaneous comparisons of many different species, solutes and temperatures, the ordinary method of tabulating the experimental data and the temperature coefficients derived from them is prohibitively cumbersome. Instead, a graphic method has been found to be very satisfactory, inasmuch as it shows at a glance both the details and the broader general relations contained in a given set of data, and at the same time it may be made accurately quantitative. The method is to draw for each species 4 lines at right angles to each other, one for every solute. On these lines the observed times of hemolysis in isosmotic solutions of the solutes, at different temperatures, are plotted logarithmically, and the points corresponding to each temperature are connected to form a quadrilateral. Under the conditions here in question, and with certain qualifications discussed elsewhere, the permeability ratio for a given 10 degree interval is approximately equal to the reciprocal of the corresponding time-of-hemolysis ratio. Because of the logarithmic method of plotting, the same distance between two points representing an interval of 10 degrees everywhere corresponds to the same Q_{10} ratio. General comparisons of the diagrams and all their details can therefore readily be made by eye. For greater accuracy an appropriate logarithmic scale permits the exact values both of times of hemolysis and of Q_{10} ratios to be read off, while a similar logarithmic scale magnified 4 times and covering a correspondingly shorter range gives directly the mean Q_{10} value for the entire 40 degree range of observations.

AUGUST 1, 1950

Orientation to heat and light during morphogenesis in the amoeboid slime molds. JOHN TYLER BONNER. No abstract submitted.

Polarized light and the orientation of bees. DONALD R. GRIFFIN.

Independent confirmation was reported of certain key points among the recent discoveries of K. v. Frisch concerning the dances and orientation of bees (*Experientia*, 1946, **2**: 397; 1949, **5**: 142; 1950, **6**: 210; and *Naturwissenschaften*, 1948, **35**: 12; 1948, **35**: 38). These included (a) the shift from the round dance to the figure eight shaped "Schwanzeltanz" as a source of sugar solution was moved from 10 to 100 meters from the observation hive, (b) a markedly greater number of turns of the Schwanzeltanz per unit time in bees returning from 100 meters as compared with others bringing pollen from much greater distances, (c) the dependence of the direction of the straight portion of the Schwanzeltanz on the position of the sun in the sky, and (d) the effect of a polaroid sheet in shifting the direction of this dance when it is being performed on a horizontal surface with only a small area of blue sky visible to the bees.

A piece of polaroid alters the brightness and color pattern of the blue sky. The possibility was therefore considered that these effects, rather than the change in plane of polarization, might account for the turning of the dances. Such an explanation, however, seems unlikely to be correct, because of the small magnitude of the differences in brightness and color within the area of blue sky which is sufficient to orient the dances and because of the reported differential brightness and color thresholds of bees (*J. Gen. Physiol.*, 1933, **16**: 773; *Zeitschr. f. vergl. Physiol.*, 1927, **5**: 762). One is forced to conclude that bees are indeed able to detect the plane of polarization of the light from the blue sky.

On the nature of Cypridina luciferin. AURIN M. CHASE.

The Japanese ostracod crustacean, *Cypridina hilgendorfii*, ejects an enzyme, luciferase, and a substrate, luciferin, into the sea water, producing a blue luminescence; probably an oxidative reaction. Luciferin also undergoes non-luminescent oxidation without luciferase (Harvey, "Living Light," Princeton University Press, 1940).

Cypridina luciferin has been partially purified (Anderson, *J. Gen. Physiol.*, 1935) and many of its chemical properties have been investigated by various workers. On the basis of these data it has been assumed to be related to the naphtho- or benzohydroquinones. The as yet unisolated luciferin of luminous bacteria has been assumed to have a similar structure, largely on the basis of absorption spectra calculated from photochemical inactivation of bacterial luminescence (Spruit, *Enzymologia,* 1949).

Because of the need for an accurate absorption spectrum of Cypridina luciferin, measurements have now been made in the visible and ultraviolet, using the Beckmann spectrophotometer and solutions of luciferin prepared from Cypridina by Anderson's method, with further treatment designed to remove lipid impurities. The resulting spectrum is unstable and changes during exposure of the solution to air. Measured in either pH 6.8 phosphate buffer or methanol, the initial spectrum shows a sharp maximum at about 265 mμ, a shoulder at about 310 mμ and a broad absorption band in the visible, centering at about 435 mμ. On standing in phosphate buffer the 265 mμ peak decreases markedly, while simultaneously a new absorption band appears at about 365 mμ. These changes are quantitatively related to the rate of disappearance from the solution of luciferin capable of giving luminescence upon addition of luciferase. The original absorption band at 435 mμ is replaced by one at about 465 mμ, which subsequently disappears.

Chase and Gregg (*J. C. C. P.,* 1949) have recently shown that nitrogen may be present in Cypridina luciferin, contrary to an earlier statement in the literature. While, therefore, the absorption spectrum of these luciferin solutions and the changes which occur during exposure to air indicate a striking similarity to naphthohydroquinone derivatives, it is possible that Cypridina luciferin may be a nitrogen-containing compound such, for example, as the pterins or pyrimidines.

Reactions catalyzed by isolated chloroplasts. ALAN H. MEHLER.

The mechanism involved in transferring the hydrogen produced in the light reaction of photosynthesis to the carbon dioxide reducing system is completely unknown. In experiments designed to separate these reactions, McAlister and Myers and Auftemgarten found a pickup of CO_2 following cessation of illumination. After illumination in the absence of CO_2, Calvin and Benson demonstrated that algae rapidly incorporated labelled CO_2 into the same compounds found labelled after short periods of photosynthesis. The latter authors ascribe their results to the photochemical formation of a long-lived general reducing compound. The following two reasons are given, however, as evidence that no such special compound exists.

First, the data of Calvin and Benson, showing fixation of CO_2 equivalent to about 6 seconds of photosynthesis, indicate a concentration of the hypothetical reducing compound of the order of magnitude of the chlorophyll concentration in the cells. No evidence for a stored reducing power was obtained with spinach chloroplasts in experiments capable of detecting reduction in amounts 1% of the amount of chlorophyll used, measuring reduction of 2,6-dichlorophenol indophenol or cytochrome c spectrophotometrically. If the reduction step of photosynthesis used a carrier present in the amounts calculated, the chloroplasts isolated might be expected to retain enough to detect.

Second, using pre-illumination and tagging techniques similar to those of Calvin and Benson, Fager has shown that essentially all of the label fixed appears in glyceric and pyruvic acids. This indicates that the reduction of CO_2 in pre-illumination experiments is limited to one step of photosynthesis.

It is concluded that pre-illumination does not result in the production of a generallized reducing power, but in changes of concentration of photosynthetic intermediates to a pattern permitting dark fixation of CO_2 in phosphoglyceric acid.

AUGUST 8, 1950

Some spectrophotometric observations on invertebrate nerves and their extracts.
A. M. SHANES AND T. J. DEKORNFELD.[1]

Suitable modifications of the Beckman spectrophotometer have permitted a study of the selective absorption of visible light by intact fin nerves of the Squid (*Loligo pealii*) and by intact leg nerves of the spider crab (*Libinia emarginata*) in the presence and absence of oxygen.

---

[1] Supported in part by a research grant from the National Institutes of Health, U. S. Public Health Service.

Observations have also been made on an ultraviolet absorbing component which appears in the sea water in contact with the nerves and which can be obtained in larger quantity by distilled water extraction. The absorption by these aqueous extracts was also examined in the visible range.

A number of distinct peaks become apparent between 540 and 570 mμ when the nerves or their extracts are rendered oxygen free; they are also seen in the presence of oxygen if the reducing agent sodium hydrosulfite is added. These changes are reversible.

Aqueous extracts prepared by soaking about 50 mgm. nerve tissue for 30 minutes in 15 ml. of distilled water give a single, sharp peak at 271 mμ, the density being about 0.5 in a 10 mm. cell. This peak, practically insensitive to changes in pH (3.0–10.3), was also observed in Loligo axoplasm kindly furnished by Drs. M. A. Jakus and A. J. Hodge of the Massachusetts Institute of Technology. The substance responsible for this ultraviolet absorption is dialyzable and is not precipitated by perchloric acid; the Biuret test proved negative. Different peaks were found with crab muscle extracts and crab blood.

Selective damage to fibroblasts by desoxycorticosterone in cultures of mixed tissues. IVOR CORNMAN.[1]

Fetal or newborn mouse heart in roller tube cultures gave rise to cells of the fibroblast type and sheets of cells resembling endothelium. Fibroblasts predominated, being the only cell type growing from 414 explants, while fibroblasts combined with endothelium grew from 144 explants, and endothelium alone from 12. DOC at 0.02–0.1 mg./ml. damaged both types of cells, but the fibroblasts reacted first, shrivelling to elongate filaments. Regeneration from the explants was predominantly endothelial. The treated cultures before exposure to DOC included 30 explants with fibroblasts alone, 28 with fibroblasts and endothelium, and 1 with endothelium alone. New growth following DOC treatment gave 11 explants with fibroblasts alone, 14 with endothelium and fibroblasts, and 34 with endothelium alone. It is concluded that cells of the fibroblast type are more quickly affected by DOC and recover less readily than the endothelial type. Cortisone added to DOC produced more severe damage of both cell types. The selective effects of DOC on fibroblasts *in vitro* can be compared with the specificity of DOC in producing fibrous lesions of a rheumatic type *in vivo*. Further tests must be made to determine whether the selective damage to fibroblasts in culture is peculiar to DOC or is merely a nonspecific steroid effect.

Ionic balance required by some marine microorganisms and effect of quaternary ammonium ions. WOLF VISHNIAC, ROY KISLIUK AND SEYMOUR H. HUTNER.

Cultivation of obligately marine microorganisms on synthetic media reveals requirements for the various properties of marine environment: osmotic pressure, high Na concentration, and ratio of Na to other ions.

The two marine agar-digesting cytophagas described by Stanier in 1941 were grown on a medium containing 3% NaCl, but other marine cytophagas which have been isolated since then required Na, Ca, Mg, and K in approximately the ratio in which these ions occur in the sea. Other marine bacteria require mainly the maintenance of osmotic pressure. *Pseudomonas drobachense* and several strains of unidentified luminous bacteria grow well in media containing at least 1.5% NaCl; however, the NaCl level may be lowered to 0.1% if osmotic pressure is maintained with 12% sucrose. An obligately marine pathogenic organism, the causative agent of "speck finger" (to be described elsewhere as *Micrococcus thjöttii*), probably behaves in a similar manner.

Experiments were conducted especially with a marine chlamydomonas, which, though largely indifferent to changes in osmotic pressure, showed a definite requirement for Na. The lower limit was usually 0.075–0.1% NaCl, depending on the balance of other ions. Mg permitted better growth at lower Na concentrations, K raised the Na requirement slightly, while in the presence of NH_4^+ high Na concentrations were required for growth.

---

[1] This work was financed by an American Cancer Society institutional grant to the Warwick Memorial for Cancer and Allied Diseases, George Washington University, Washington, D. C.

At limiting NaCl concentrations the addition of 0.05–0.1% tetraethylammonium bromide (Et_4NBr) increased growth of the chlamydomonas by a factor of 3–5; Et_4NBr also permitted growth at a slightly lower Na level (0.05% NaCl). At best there is therefore only a partial replacement of Na by Et_4N^+ for the growth of marine organisms, in contrast to the claims of de Nó concerning nerve excitation. Synaptic blocking by Et_4NBr is probably an effect independent of any relationship to Na.

The control of elongation in the hypocotyl of the kidney bean. WILLIAM P. JACOBS.

Two regions of maximal growth were found in the hypocotyl of kidney bean seedlings. The first region was dominant during the first few days of seedling growth, the second region during the later growth. By measuring the growth of isolated sections, it was shown that the second region can grow before it normally does in the plant if it is given an external supply of auxin and sucrose. By direct test using the *Avena* bioassay, it was shown that the second region contains negligible amounts of diffusible auxin, that auxin can not be transplanted acropetally through any region of the hypocotyl, but that it can be transported basipetally. Hence, auxin for the growth of the second region probably comes from above. It has long been known that cotyledons and/or the epicotyl are sources of diffusible auxin. For auxin from the cotyledons and epicotyl to reach the second region it must pass through the first region. Therefore, the transport properties of the first region were studied. It was found that no auxin was transported across the first region in the 3-day or earlier stages of seedling growth, that an increasing amount could be transported in successively older seedlings. Hence, it seems probable that the second region, needing auxin to grow, is being limited in its growth by the auxin-transport ability of the region standing between it and its auxin supply. This conclusion is confirmed by the very high correlation coefficient (0.94) between the growth of the hypocotyl and the amount of auxin which can be transported through the first region.

AUGUST 15, 1950

The sensitivity to light, reactions to shading, pigmentation, and color change of the sea urchin, Diadema antillarum Philippi. NORMAN MILLOTT.

That certain sea urchins react to changes in light intensity has long been known. The black urchin *Diadema antillarum,* common around the coast of Jamaica, responds by vigorous spine movements to both increase and decrease in intensity, but as the responses to the latter are more marked and regular, they have been studied exclusively.

The response has a latent period of 0.6–1.4 seconds and is oriented, most of the spines converging toward the object casting the shadow. A sharp shadow is not essential, diminution in intensity of illumination is sufficient to elicit the response, provided it occurs rapidly. Removal of a section of the nerve ring, although affecting the tone of the spine muscles, does not abolish the response to shading, which can be obtained in isolated pieces of test, provided that the radial nerve strand and its lateral branches remain intact. Stimulation by isolated light spots 0.5 mm. and 1.5 mm. in diameter shows that the spines are insensitive. The whole surface of the test and peristome is sensitive, but not equisensitive, the order of sensitivity being ambulacral margins → ambulacral center → interambulacrum. There is a significant relationship between sensitiveness and pigmentation, darker regions being invariably more sensitive than paler and the order of sensitiveness and degree of pigmentation correspond exactly. The receptors are located outside the test and have a wide distribution, the latter discovery excluding the possibility of structures which have previously been described as eyes in allied forms, from being the sole photoreceptors. The extensive superficial nerve layer may therefore be stimulated directly by changes in light intensity, and the response has the characteristics of a reflex with the centers in the radial nerve strands.

Diadema shows a color change which is most marked in young forms, being lighter by night, certain parts of the test becoming completely white and forming a pattern, which, during intermediate phases of the color change, appears a brilliant blue, due to dispersion effects of the receeding black pigment. As the animal becomes lighter its sensitivity to shading undergoes a marked increase. The color change is rapid (about 1½ hours is required for the full range) and largely due to changes in large skin chromatophores, which are highly branched in the expanded phase and globular in the contracted; their behavior is independent of the central nervous system and localized expansion of the contracted chromatophores can be elicited by projecting a light spot on them. Despite the obvious correspondence between depth of color and light intensity, there is an underlying, diurnal rhythmic color change, for forms kept in total darkness become slightly darker by day.

The black pigment gives the reactions characteristic of melanin, and may be formed in the amoebocytes of the coelomic fluid which darken on exposure to air, especially after irradiation with ultraviolet and if a trace of catechol be added to the coelomic fluid. The coelomic fluid will oxidise catechol and "dopa" *in vitro*, but feebly, at pH 7.4 and 30°C.

The fact that a more rapid oxidation of "dopa" is achieved by a water extract of the amoebocytes, separated by centrifuging from the coelomic fluid, suggests that an inhibitory factor to phenolase activity exists in the coelomic fluid. The rapidity with which 0.01% methylene blue injected into the coelom is reduced, suggests that the inhibitory factor might be associated with a system of low oxidation-reduction potential, while the abolition of the inhibitory effect by methyl alcohol is somewhat suggestive of a dehydrogenase.

Photorecovery after ultraviolet radiation in amphibian larvae. HAROLD F. BLUM AND MARGIE M. MATHEWS.

Photorecovery after ultraviolet radiation was demonstrated in the larvae of *Amblystoma*. The larvae were exposed five days per week to sub-lethal doses of intense ultraviolet radiation ($\lambda\, 0.23\,\mu$ to $0.313\,\mu$). When the larvae were illuminated with "visible" radiation from fluorescent lamps after the exposure to ultraviolet, they survived longer than if kept in the dark. Thus, visible radiation enhances recovery from the effects of the ultraviolet. Larvae illuminated with visible radiation for five hours immediately after each dose of ultraviolet, survived longer than larvae illuminated with visible radiation for five hours just prior to dosage with ultraviolet. The latter survived longer than larvae kept in the dark. This shows that recovery is not yet complete 19 hours after the exposure to ultraviolet, and that illumination with visible radiation is still effective at that time. The longest wavelength causing photorecovery is about $0.5\,\mu$. The short wavelength limit is not yet established, but wavelengths in the near ultraviolet are effective. Thus, the wavelengths for photorecovery are restricted to the same spectral region as "photoreactivation" in fungi and bacteria (Kelner; Dulbecco), and photorecovery of cleavage rate in sea urchin's eggs (Blum, Loos and Robinson). The prolonged recovery process, and the absence of effect of visible light on the normal organisms are also paralleled in the above instances. All appear to represent the same fundamental process; the repair of effects of ultraviolet radiation by a specific range of wavelengths extending from the very near ultraviolet to the blue. The present instance seems to be the first described in a vertebrate, extending the known distribution of this phenomenon in the animal kingdom.

Action of alcohol dehydrogenase on vitamin A. ALFRED F. BLISS.

Retinene is a fat-soluble yellow substance first obtained from the photolabile visual pigment, rhodopsin (visual purple). It has been identified by Morton as vitamin A aldehyde, and is reduced to vitamin A alcohol by various tissues, including the retina and intestinal mucosa. Wald has shown that coenzyme I is required for the reaction in the retina. We have shown that the reaction occurs in solutions of bleached visual purple and is reversible (*Biol. Bull.*, 1949, **97**: 221), both in isolated retinal rods and in liver extracts containing alcohol dehydrogenase. The reversibility of this step shows that the regeneration of visual purple from vitamin A can procede via vitamin A aldehyde.

In the present experiments, the equilibrium constant of the dehydrogenation of vitamin A by coenzyme I was determined with the aid of alcohol dehydrogenase obtained from horse liver by a modification of the method of Bonnichsen and Wassen (*Arch. Biochem.*, 1948), involving fractionation by ammonium sulfate between 50 and 60 per cent saturation and by alcohol. A linear relation between log k and pH was obtained, as found by Racker (*J. B. C.*, 1950) for ethyl alcohol. The dehydrogenation of vitamin A was much more extensive than that of alcohol, the respective k_H for each being $3.3 \cdot 10^{-9}$ and $1.15 \cdot 10^{-11}$.

The regeneration of visual purple from vitamin A aldehyde in solutions of bleached visual purple was dependent on a thermolabile factor extracted from the melanotic pigment layer of the frog eye by dilute NaCl. An association of the factor with the protein fraction was indicated by its resistance to dialysis and by precipitation with ammonium sulfate and alcohol, with recovery of about 50 and 25 per cent, respectively, of the initial activities (*Fed. Proc.*, 1950).

AUGUST 22, 1950

Population dynamics in Escherichia coli. LILLIAN K. SCHNEIDER.

The mutation from histidine dependence (h-) to histidine independence (h +) in the bacterium, *Escherichia coli*, has been shown to be spontaneous by application of the Luria and Delbruck variance analysis. The growth rate and final level of growth of h − on medium with optimal histidine (25γ/cc.) are the same as h + with no histidine (H −) and with 25γ histidine/cc. (H +). Mixtures of h − and h + grown on H + show no shift in ratio after only twelve generations. Definite shifts occur over a greater number of generations.

Cultures of pure h − bacteria, passed through hundreds of generations by serially transferring 0.5 cc. of a fully grown culture to 50 cc. of fresh H + every twelve hours, show an increasing number of h + back mutants until an equilibrium ratio of $1\text{h}+/10^6\text{h}-$ is attained. This equilibrium has been maintained for more than four hundred generations in several different experiments. The forward and back mutation rates are not sufficient to explain the ratio observed. A selection pressure of 0.02 against the h − has been calculated.

Cultures started at high h +/h − ratios, by making artificial mixtures, show a long period with no shift and then a rapid drop in h + ratio until the same equilibrium value as above is reached. This has been shown to be due to the selection of a fast growing h −. The equilibrium is reached with the appearance of a fast growing h + from these fast h − bacteria. An unusual feature of this situation is that although a new h − is selected for, the same equilibrium value is reached. Independent measurement of the selection pressure against h + and a more precise definition of the selection are now being studied.

Heterokaryosis between opposite mating types of Neurospora crassa. SAMSON R. GROSS.

Heterokaryosis between like mating types in *N. crassa* is a well known phenomenon; its occurrence between opposite mating types has been subject to controversy. To demonstrate heterokaryosis between opposite mating types four laboratory stocks were used: lysineless albino *a* (7S2a), pantothenicless albino *a* (26a), pantothenicless pink *A* (5531A), and lysineless pink *A* (4545A). Two heterokaryons were prepared by mixing conidia of 26a and 4545A, and 7S2a and 5531A on minimal Fries 2% agar slants and incubated at 25° C. To prove that heterokaryosis rather than syntrophy alone had occurred, single hyphae were isolated. Fifty per cent of the isolates proved viable on minimal medium. They crossed freely with both *A* and *a* tester strains and also *selfed*. Plating macroconidia obtained from these cultures on sorbose medium (a medium causing restricted growth) supplemented by pantothenic acid or lysine, indicated the presence of both nuclear types.

The growth rate of these heterokaryons is slow and irregular. Studies of the ratios of the homokaryotic conidia in this heterokaryon indicate that the slow rate of growth may be due to an alteration of the ratios of the two nuclei so that the organism is, in effect, growing on a limited amount of growth factor. Ratios of homokaryotic conidia obtained from heterokaryons between like mating types are from two to eight pantothenicless to one lysineless. If these conidial ratios reflect the ratios of nuclei in the mycelia then there is an effective range of ratios of pantothenicless nuclei to lysineless capable of maintaining the organisms at a normal growth rate. In the heterokaryon 7S2a : 5531A the ratio of conidia is found to be 20 to 80 to 1 and for the 26a : 4545A heterokaryon 400 to 1200 pantothenicless to one lysineless.

The pantothenicless conidia, then, are normally in excess when the mating types are similar. They are more strongly in favor of the pantothenicless when the heterokaryon is between opposite mating types; and there is greater variation in the nuclear ratios depending upon whether the *a* is part of the pantothenicless genotype rather than the lysineless. We may infer from this fact that attempts to study dominance relationships in heterokaryons should be approached with caution since nuclear ratios often may be determined by factors other than the auxotrophic mutants under investigation.

The homology patterns of induced lethal mutations in Neurospora crassa. K. C. ATWOOD.

Ultra-violet induced lethal mutations were obtained and preserved as heterokaryons by a method previously described (*Biol. Bull.*, 1949, **97**: 255). In an attempt to estimate the number of identical mutations in a series of 156 independently induced lethals, each mutant was tested for homology with every other mutant. This was done by forming a heterokaryon between two heterokaryons each carrying a lethal in its amycelial component. The conidia of this secondary heterokaryon were then streaked on sorbose medium, and the appearance of amycelial colonies in the streak where none appeared in control streaks of the primary heterokaryons was taken as a certain indication that the lethal mutations were not homologous. Since the secondary heterokaryons were not forced, the cases where amycelial colonies failed to appear can be interpreted in two ways: either one of the component primary heterokaryons excluded the other, or the lethal mutations in the primary heterokaryons were homologous. Of 1746 tests made to date within five groups of 14, 16, 29, 49, and 47 mutants, 1488 or 85.3 per cent showed the non-homologous reaction.

To the extent that homology as determined by this represents simple allelism, one would expect that two homologous mutants would show identical patterns of homology when tested against other mutants within a group. Actually, this type of internal consistency was not found. None of the mutants showed identity of reactions within the group.

Certain mutants, however, appeared to be simultaneously homologous with a large number of others which were mutually non-homologous. These can be interpreted as large chromosomal deficiencies which overlap many smaller, separate deficiencies or many loci. Assuming that there are deficiencies, the minimum number of loci which must be postulated to account for the pattern was found to be slightly greater than the number of mutants tested.

The alternate interpretation, *i.e.*, that homologous reactions are false, implies in all groups a number of loci equal to the number of mutants tested. In short, no matter how the pattern of homology is interpreted, the conclusion is forced that the number of loci in Neurospora is very large.

Comparison of the base composition of nucleic acids of nuclei and cytoplasm of different mammalian tissues. ALFRED MARSHAK.

After separation of nuclei and cytoplasm and extraction with cold TCA and with fat solvents, aliquots were digested with $HClO_4$ and the nitrogenous base content determined by paper chromatography and spectrophotometery. Other aliquots were subjected to the Schmidt-Thannhauser procedure and the bases determined on the DNA and the PNA-nucleotides so separated. Recovery in DNA and RNA agreed with that of the unfractionated materials within 1–4% for all bases except guanine where a single 10% deviation was found. Previous experiments showed that recovery of bases from $HClO_4$ digests of nucleic acids was quantitative (96–104%).

The base ratios of DNA from nuclei of all tissues were the same within the limits of error. As a group the nuclear PNA's (N–PNA's) had base ratios strikingly different from those of the cytoplasmic PNA's (C–PNA's). N–PNA of thymus was exceptional in having a very low cytosine content. Heart C–PNA had no detectible uracil, (The value given in the table is for some unidentified substance with the same Rf as uracil, but with a different absorption spectrum.) The structural difference between N–PNA and C–PNA may be correlated with the difference in function of the two nucleic acids previously reported. The marked differences in the base ratios of the N–PNA's of different tissues implies that cellular differentiation involves chemical change of nuclear constituents as well as cytoplasmic changes. The low adenine content of the C–PNA's suggests that the free adenine nucleotides of the cell may be liberated on the conversion of N–PNA to C–PNA, since previous experiments show that the former may be the precursor of the latter, while the reverse is not possible. The guanine-

TABLE I

Ratios of mols of purines and pyrimidines with adenine as unity

	Guanine	Cytosine	Thymine	Uracil
Nuclei				
DNA				
Liver	0.89	0.85	1.04	—
Thymus	0.89	0.79	0.99	—
Kidney	0.79	0.78	1.00	—
Heart	0.82	0.82	1.01	—
PNA				
Liver	1.00	0.39	—	0.45
Thymus	0.88	0.09	—	0.17
Kidney	0.92	0.25	—	0.59
Heart	0.92	0.19	—	0.29
Cytoplasm				
Liver	1.83	1.22	—	1.12
Thymus	1.88	1.44	—	0.89
Kidney	1.78	1.25	—	0.94
Heart	1.20	0.77	—	0.03 (?)

adenine ratios of the C–PNA's may be accounted for by the loss of one mole of adenine from the N–PNA's.

GENERAL SCIENTIFIC MEETINGS

AUGUST 29–30, 1950

Oxidative phosphorylation by a cell-free, particulate, enzyme system derived from fertilized Arbacia eggs. PHILIPP STRITTMATTER, A. K. KELTCH, C. P. WALTERS AND G. H. A. CLOWES.

During the summer of 1949 a cell-free, particulate, oxidative, phosphorylating system was obtained from unfertilized but not from fertilized Arbacia eggs. A comparison was made between the action of a series of nitro- and halophenols on the respiration and cell division of fertilized Arbacia eggs and on the respiration and phosphorylation of the cell-free, particulate system obtained from unfertilized Arbacia eggs. Since any comparison of these effects rests on the assumption that the cell-free, particulate systems derived from unfertilized and fertilized Arbacia eggs have similar phosphorylating mechanisms, it appeared desirable to make further attempts to obtain an oxidative, phosphorylating system from fertilized Arbacia eggs.

When the procedure found successful in preparing the particulate system of unfertilized Arbacia eggs (*Biol. Bull.*, 1949, **97**: 242) was attempted with fertilized eggs, it was found necessary to force the suspension through the needle fifteen to twenty times in order to rupture the egg membranes, thereby resulting in complete disintegration of the cell contents. As a result of this more violent treatment or perhaps some less obvious variation, the supernatant was totally inactive as a phosphorylating system. Little success was achieved by varying the potassium chloride and citrate content of the homogenizing solution, adding cysteine or glutathione to protect sulfhydryl groups or heparin to inhibit coagulation, or by maintaining a temperature as close to 0° C. as possible.

Finally, a more drastic procedure was employed. The fertilized eggs, after two washings with sea water and centrifuging, were chilled and shaken for less than a minute with a cold, aqueous solution containing 0.72 *M* urea and 0.125 *M* NaCl. They were subsequently washed three times with a cold, buffered, isotonic NaCl solution. The fertilized eggs thus treated still exhibited normal cell division when returned to sea water, but the cell membranes were more friable and the eggs could be broken down by forcing them through the syringe three times. When centrifuged at a low temperature, a cell-free particulate system was obtained from the fertilized Arbacia eggs which gave phosphorylation ranging up to 30 per cent ester formation from the inorganic phosphate present when succinate was used as substrate and over 20 per cent with glutamate and α-ketoglutarate. The results obtained with the substituted phenols, although not yet extensive, indicate that the effects on phosphorylation in the fertilized and unfertilized egg extracts are identical.

The concentrations of 4,6-dinitrocresol and 2,4,5-trichlorphenol required to give a 50 per cent block to phosphorylation of the cell-free homogenates obtained from unfertilized and fertilized Arbacia eggs were identical within the range of experimental error, being 7.9×10^{-7} *M* and 5.6×10^{-6} *M*, respectively, for homogenates from unfertilized eggs and 10^{-6} *M* and 4×10^{-6} *M*, respectively, for the homogenates obtained from fertilized eggs at pH 7.2.

Similar experiments were started with unfertilized and fertilized eggs of *Mactra solidissima*, which have extremely tough membranes. On account of lack of material, further experiments on Mactra have been postponed until next season.

Action of a series of nitro- and halophenols on the respiration and cell division of Mactra solidissima. A. K. Keltch, Philipp Strittmatter, C. P. Walters and G. H. A. Clowes.

A series of nitro- and halophenols which block cell division and stimulate respiration of Arbacia also block cell division and stimulate respiration of Mactra, while those that are inactive on Arbacia are also inactive on Mactra. This block to division is, as in the case of Arbacia, fully reversible over a wide range of concentrations. The concentrations of nitro- and halophenols required to effect a fifty per cent block to cell division were generally four to eight times the concentrations required to exert similar effects on Arbacia. The concentration of substituted phenol which gives the maximum increase in respiration corresponds very closely with the concentration which produces the initial cell division block.

The respiratory stimulation effected by these nitro- and halophenols on Mactra is, when expressed as per cent increase over untreated eggs, double the increase effected by the same compounds on Arbacia. For example, 2,4-dinitrophenol increases respiration of (a) fertilized Arbacia eggs 290 per cent and (b) fertilized Mactra eggs 650 per cent.

The experimental procedure followed was the same as that used for Arbacia eggs (*J. Gen. Physiol.*, 1936, **20**: 145) except that the substituted phenols were added to the eggs at 40 minutes after fertilization instead of 25, thereby allowing time for maturation of the Mactra eggs. The effects exerted by a series of substituted phenols on the respiration and cell division of Mactra are reported in the accompanying Table.

Conc. reagent	2,4-dinitrophenol		4,6-dinitrocresol		4,6-dinitrocarvacrol		2,4-dinitrothymol		2,4,5-trichlorophenol	
	O_2 use	Div. per egg	O_2 use	Div. per egg	O_2 use	Div. per egg	O_2 use	Div. per egg	O_2 use	Div. per egg
Moles per l.										
0	63	3.6	64	3.6	65	3.7	55	3.8	58	3.7
1×10^{-6}										
2×10^{-6}							100	3.8	67	3.7
4×10^{-6}					124	3.7	113	3.8		
8×10^{-6}	81	3.5					162	3.0	94	3.7
16×10^{-6}	96	3.6	169	3.6	243	3.7	95	0.0	117	3.7
32×10^{-6}	104	3.6			314	2.5	77	0.0	220	3.7
64×10^{-6}	161	3.6	349	0.3	312	0.0	23	0.0	450	0.0
128×10^{-6}	218	3.6	436	0.0	224	0.0	20	0.0	138	0.0
256×10^{-6}	320	3.4	410	0.0	140	0.0	13	0.0	115	0.0
512×10^{-6}	407	0.6								
1024×10^{-6}	400	0.0	116	0.0	73	0.0			88	0.0

Action of dinitrocarvacrol and dinitrothymol on respiration of fertilized Arbacia and Mactra eggs and certain mammalian tissues and cell-free, particulate, phosphorylating systems. G. H. A. CLOWES, A. K. KELTCH, PHILIPP STRITTMATTER AND C. P. WALTERS.

The effects exerted by varying concentrations of dinitrocarvacrol and dinitrothymol on the respiration and cell division of fertilized Mactra eggs are presented in the last two sections of the Table in the preceding paper. In both cases a reversible block to cell division is effected at a given concentration of the reagent and a peak of respiration is observed at or just beyond the highest concentration at which cell division still occurs. These effects are in sharp contrast to those observed with Arbacia. While dinitrocarvacrol induces in fertilized Arbacia eggs the usual marked rise in respiration, reaching a peak at a point corresponding approximately with that at which a 50 per cent block to division is induced, dinitrothymol, while giving a reversible block to cell division in the same manner as dinitrocarvacrol, exerts no stimulating effect whatever on respiration, but induces a sharp fall in oxygen consumption from the beginning.

The cell-free, oxidative, phosphorylating extracts of unfertilized Arbacia eggs give results corresponding with those obtained with the intact fertilized eggs. Both dinitrocarvacrol and dinitrothymol induce reversible blocks to phosphorylation at concentrations corresponding with those required to block cell division. Dinitrocarvacrol, with rising concentration, causes a slight increase in respiration, followed by a fall, while dinitrothymol, with rising concentration, causes an abrupt reduction in respiration, as in the case of the intact eggs.

Unfortunately, we have not thus far had an opportunity to test cell-free, particulate systems derived from Mactra eggs. Cell-free, particulate systems derived from mammalian kidney and liver exhibit with both isomers a block to phosphorylation, with a corresponding slight, temporary rise in respiration.

From previous experiments the conclusion has been reached that adsorption of the un-ionized substituted phenol molecule on the surface of the oxidative, phosphorylating enzyme is probably responsible for the reversible block to cell division and the corresponding fall in respiration in fertilized Arbacia eggs. Some evidence has been presented indicating that the initial stimulation of respiration may be attributable to the substituted phenol ion. The experiments reported above support this viewpoint. The fact that a series of nitro- and halophenols block cell division and respiration at far lower concentrations in Arbacia than in Mactra suggests that components of the surface of the phosphorylating enzyme in Arbacia have a greater affinity for the un-ionized substituted phenol molecules than do corresponding components of the surface of the phosphorylating enzyme in Mactra. The peculiar behavior of dinitrothymol in depressing respiration in Arbacia eggs and cell-free, phosphorylating systems derived from these eggs, while stimulating respiration in Mactra eggs and in cell-free mammalian kidney and liver phosphorylating systems, may well be attributable to steric hindrance due to the presence of the isopropyl group in the ortho position to the phenolic hydroxy group in dinitrothymol. This might interfere with the action of the ion, particularly in the case of Arbacia, in which the affinity of the un-ionized molecule of the substituted phenol for components of the surface of the Arbacia phosphorylating mechanism is presumed to be greater than that for the corresponding components of the Mactra phosphorylating system.

The complete reversibility of the cell division block in both Arbacia and Mactra, even after exposure of the eggs for a considerable period to concentrations 1000 fold those required to block division, lends support to the view that the attachment of the un-ionized substituted phenol molecule to surface components of the phosphorylating enzyme is probably due to hydrogen bonding and van der Waal effects.

Some observations on phosphate uptake by Tetrahymena geleii. JAY S. ROTH, ALFRED M. ELLIOTT AND ROBERT L. HUNTER.

Tetrahymena geleii E was grown in a synthetic medium containing 100γ of phosphate labeled yeast nucleic acid per ml. of medium. The labeled nucleic acid was prepared by growing yeast in the presence of P^{32} using a modification of the method of DiCarlo (*J. Biol. Chem.*, 1949, **180**: 329). The only other source of phosphorus was protogen, an essential growth factor for the protozoa which contributed 2.04γ of phosphorus per ml.

The uptake of phosphorus from the yeast nucleic acid was determined by following the change in activity of the supernatant and in the washed protozoa. Of 7.65γ of nucleic acid phosphorus per ml., 3.52γ or 46% was taken up during the first 60 hours. The rate of uptake was constant from 12–60 hours and became zero after that time. The number of organisms reached a maximum at 60 hours. Inorganic phosphate in the medium was also followed and found to increase during the first 24 hours of growth, followed by a slow decline. This gives further support to the observations that *Tetrahymena geleii* produces an enzyme capable of splitting phosphate from nucleotides or nucleic acid, into the medium.

Double refraction of flow studies on Desoxypentose nucleic acid. Alexander Rich.

Until recently, the double refraction observed in solutions subjected to a velocity gradient could be used to measure particle size only for the limiting case of extinction angle approaching 45°, *i.e.*, at low velocity gradients (*Adv. in Coll. Sci.*, 1942, **1**: 269). It is now possible to make measurements for all values of the extinction angle and translate these results into particle size (Sheraga, Edsall and Gadd, to be published). By this means, one can ascertain whether a given solution acts as a mono- or polydisperse system. In addition, the influence of concentration can be studied, thereby obtaining a measure of molecular interactions.

In this study, the desoxypentose nucleic acids (DNA) were prepared by the method of Gulland *et al.* (*J. C. S.*, 1947, **11**: 29) from the testis and spermatophores of the squid, *Loligo pealii.* The N/P ratio was 1.70. All double refraction of flow (DRF) experiments were carried out at 24°C.

The effect of concentration on the apparent rotary diffusion constants (θ) of DNA was studied in a 2 M NaCl solution. At concentrations of .15% DNA, the deviations of the extinction angle-velocity gradient curves from that expected of a monodisperse, non-interacting solution were marked. However, on reducing concentration to .06 or .04%, the curves rapidly approached the theoretical relationship for a monodisperse system. This indicates that the DNA preparation is homogeneous as respects particle length in the region studied for velocity gradients up to 500 sec.^{-1}.

An attempt was made to calculate the rotary diffusion θ for infinite dilution by extrapolating to zero concentration. This could not be done directly, since the change in θ becomes very large at low concentrations. θ is inversely proportional to an inner frictional constant ζ, which is a measure of the torque required to maintain the molecule rotating with unit angular velocity. It was expected that ζ should decrease with a fall in concentration, and on plotting concentration vs. $1/\theta$ a straight line was obtained.

The remarkable linearity of concentration and $1/\theta$ is equivalent to stating that the inner frictional constant ζ is composed of an intrinsic part (ζo) and a concentration dependent portion (ζc), *i.e.*, $\zeta = \zeta o + \zeta c$. The slope of this line is an interaction constant

$$\left[\frac{d\left(\frac{1}{\theta}\right)}{dc} \,\alpha\, \frac{d\zeta c}{dc} = K\right]$$

characteristic of the parameters of the solution, *i.e.*, pH, ionic strength, etc., and can be used as a quantitative measure of molecular interaction.

On extrapolating to infinite dilution, DNA in 2 M NaCl has a rotary diffusion constant of 72, and a particle length of 6650 A° (for axial ratio = 200).

The kinetics of denaturation of several proteins. Robert C. Warner and Milton Levy.

The kinetics of denaturation of several proteins have been examined as a function of pH and temperature. Denatured protein was determined as that material which became insoluble at the isoelectric point of the protein. Although there are numerous reports in the literature of first order denaturation reactions, and first order kinetics were found for the denaturation of ricin (*J. Biol. Chem., In press*), only ovalbumin of the four proteins studied proved to be of this type. Bovine serum albumin, lactoglobulin and horse methemoglobin all showed characteristics of higher order reactions.

The data for ovalbumin are most simply interpreted on the assumption that the protein consists of two components which denature at different first order rates.

Serum albumin denaturation is characterized by a lag period during which no insoluble protein is formed, followed by the formation of precipitable protein approximately by a second order process. The entire reaction is best fitted by an initial first order reaction leading to the formation of a soluble intermediate which then polymerizes to give an insoluble product. The slope of a second order plot is proportional to the initial protein concentration at alkaline pHs, but in acid solution a greater dependence on initial concentration is found.

Lactoglobulin shows the same sort of kinetics as serum albumin in acid solution, but the lag period disappears at alkaline pHs. The slopes of the second order plots are dependent on the initial protein concentration.

Horse methemoglobin denaturation is best fitted by a second order plot without a lag period. The experiments were all done at a single protein concentration.

For consideration of the variation of rate with pH the second order slopes were compared at a constant initial protein concentration.

The effect of pH upon the rate of denaturation of several proteins. MILTON LEVY AND ROBERT C. WARNER.

By working at constant protein concentration, in those cases where this has a significant effect on rate, psuedo constants have been obtained for a wide range of pH values for the rates of denaturation (insolubilization) of bovine serum albumin, horse methemoglobin, lactoglobulin and ovalbumin. These constants when plotted for a single temperature as logarithms against the pH show that only a few acid-base groups are significant in determining the rates in spite of the many such groups present in the proteins. Bovine serum albumin shows minimal rates at pH 2.5 and 6.5 at 66° with a very high intervening maximum centering at pH 4. Between pH 2.5 and pH 4 the temperature coefficient of rate is very small, whereas at all other pH values it is large. The high maximum at pH 4 could explain the finding (attributed to reversibility) that solutions of serum albumin heated in acid solution and neutralized while hot, precipitate completely; whereas those which are first cooled to room temperature and then neutralized are nearly completely soluble. The data tend to confirm the hypothesis that prototropic hydrogen bonds are much more important elements in the structure of native proteins than are salt bridges formed between positive and negatively charged groups by virtue of coulombic forces.

Some physical constants of the Fundulus egg. OTTO GLASER.

An adequate back log of physical data on developing *Fundulus heteroclitus* is difficult to find or establish. Even after mechanical or chemical removal of the outer "jelly," fibrous and sticky, weight/volume ratios without further correction may be irreconcilable with the fact that, in sea water, these eggs invariably sink.

Absurdities are completely eliminated by the flotation method. Four volumes of sea water plus one volume M sucrose can be easily adjusted so that the eggs are just afloat. Density is then given by the Westphal balance. Thirty-six experiments averaged a floating density of 1.0464 for the capsules with eggs but without jelly. This value averages 2.25% more than the average of the corresponding sea waters. Within datum, we can approximate closely the average flotation densities without flotation.

Our chief interest is the egg inside the capsule. Both, we assume, are spheres. Frequently this agrees with the perpendicular diameters measured by ocular micrometer; sometimes the assumption is only the simplest approximation.

The difference between the two volumes is the volume of the highly variable perivitelline space. If perivitelline liquid is essentially sea water, the density of the latter enables us to calculate the weight of the intracapsular fluid. Subtraction from total capsular weight, known by flotation or direct weighing, gives us the weight of the egg proper. Egg-densities average 1.0537. An average based only on extremes comes to 1.0530. Egg weights vary from 2.38 to 3.47 mgs.; volumes, from 2.26 to 3.30 mms^3. Densities based on these extremes average 1.0523. Average egg-densities during the first four days of development are 0.0063 greater than during the days just prior to hatching. We may doubt if this difference is significant.

Temperature alteration of carbamate narcosis of dividing Arbacia eggs. IVOR CORNMAN.[1]

The amount of ethyl carbamate (urethan) necessary to produce 20–40% retardation of Arbacia egg cleavage varies with temperature. At 12°, 16° and 20°C., the effective concentrations of urethan are 2.5, 5 and 6.1 g./L, respectively. At 21°, 25° and 29°C. the doses are 7, 5, and 4 g./L. Urethan is least effective at 20°–21°C., and becomes more effective if the temperature is raised or lowered beyond this point.

These opposing trends indicate that two mechanisms are involved and that urethan acts upon one or both to produce the same visible result. Urethan narcosis both at high and low temperature is characterised by inhibition of cytoplasmic cleavage while the nuclear processes continue and eventually produce four blastomeres by means of two simultaneous cleavages. Increased effectiveness at high temperatures is consistent with the proposal that urethan facilitates denaturation of protein. Chemical combination has been proposed as a mechanism by which urethan exerts some of its effects. Combination would be enhanced at low temperatures and so could explain the increased narcosis below 20°C. A probable site of combination on the urethan molecule is the amino group, but when one or both of the amino hydrogens are replaced with alkyl groups, the effectiveness of the carbamate is increased. Ethyl-N,N-diethyl carbamate, $(C_2H_5)_2NCOOC_2H_5$, is four times as potent as ethyl carbamate, but like ethyl carbamate is least effective at 20°–21°C. and selectively inhibits cytoplasmic cleavage. Propyl and butyl carbamate are also more active than ethyl carbamate. Inasmuch as substitution on either or both ends of the urethan molecule augments its inhibition of cleavage, the amino or ester linkages appear to be unlikely sites for the narcotic to combine with cellular components. Adsorption of the intact molecule appears more probable.

Isotonic solutions for the erythrocytes of the smooth dogfish, mackerel and tautog. JAMES W. GREEN AND JOSEPH F. HOFFMAN.

As an outgrowth of studies on the permeability of fish red cells an investigation was made to determine what concentrations of salt solutions were isotonic with the red cells of the smooth dogfish, mackerel and tautog. Previous work in this field had been chiefly confined to freezing point depression measurements of whole blood or serum and the calculation from such values of isotonic solutions. Four methods were used in the present study for the determination of isotonicity: (1) freezing point depression, (2) hematocrit, (3) volume changes by densimeter, and (4) hemoglobin concentration changes. In general the isotonicity of each sample of blood was determined by each of the 4 methods. Blood was removed from the caudal vessels of the smooth dogfish by syringe, from the mackerel and tautog by cutting the gills or by heart puncture. For tautog and mackerel it was necessary to pool the blood obtained from several fish. Both oxalated and defibrinated blood was used. The isotonic concentrations were the same from blood drawn from gills or heart which indicates that negligible amounts of sea water were obtained with gill blood.

The following average isotonic values were obtained for NaCl expressed as moles. The measure of variation is shown by the standard error.

Fish	Hematocrit	Hemoglobin	Densimeter	ΔT_f
Dogfish	0.34±.008	0.35±.02	Ca. 0.53	0.57±.003
Mackerel	0.27±.007	0.26±.005	0.26±.004	0.33±.004
Tautog	0.21±.007	Ca. 0.23	0.26±.007	0.27±.008

Further dogfish red cell studies were made with NaCl-urea solutions (22.28 gm. NaCl and 21.4 gm. urea per liter), calculated as 0.56 M NaCl. On this basis 0.47 M ± .004 was isotonic by the hematocrit; 0.51 ± .009 isotonic by hemoglobin analysis and 0.52 ± .005 isotonic by the densimeter.

---

[1] Aided by a grant from Bache Fund of the National Academy of Sciences and by an American Cancer Society institutional grant to the Warwick Memorial for Cancer and Allied Diseases, George Washington University.

Further studies on the action of a cytoplasmic component on jelly coat and membrane formation in Arbacia punctulata. Alberto Monroy and John Runnström.

As a continuation of previous work of the present authors (*Ark. f. Kemi*, 1950, 2) the precipitation of the jelly coat of intact eggs under the influence of egg extracts has been investigated. 1 *M* KCl extracts of frozen dried eggs of *Arbacia punctulata*, dialysed against sea water, have a strong precipitating action on the jelly coat both *in vivo* and *in vitro*. The extracts were fractionated by ultracentrifugation and the separated fractions controlled electrophoretically. After removal of a first fraction sedimenting at 25,000x g, ultracentrifugation at 155,000x g leads to the formation of two layers: a heavily pigmented sediment and a slightly yellowish supernatant. The activity is entirely confined to the sedimented fraction. In this latter two components can be recognised on electrophoresis. Attempts at further purification have so far failed. Thrombin has also been found to be active in precipitating the jelly coat, both *in vivo* and *in vitro*. This, however, occurs only when the salt concentration of the medium is lowered. The effect of thrombin is completely counteracted by heparin. The cytoplasmic component isolated according to the procedure indicated above corresponds to the "antifertilizin" (AF) of the egg. AF inhibits the agglutinating action of "fertilizin." It was often observed that AF not only precipitates the jelly coat but also renders the vitelline membrane more refractive. Eggs were fertilized in an 0.0026 *M* solution of Jodoso-benzoic acid in sea-water. The membranes formed soon become more refractive than normally. An increased retardation can be demonstrated in the polarization microscope. Gradually also a coagulation of the surrounding jelly coat may occur. These changes are correlated with an increased release of pigments to the perivitelline space. Observations on previously centrifuged eggs show that the active agent is released from the pole containing the pigment granules. Our data when considered together suggest that AF is released from the pigment granules, subsequent to the attachment of the spermatozoon, and that it exerts a *coagulating* action on the fertilization membrane.

The exchange of radioactive calcium by unfertilized and fertilized Arbacia punctulata eggs with or without jelly coats. F. Hermann Rudenberg.[1]

During measurement of calcium uptake by sea urchin eggs, designed to unearth any temporal correlation which may exist with observed structural changes, a considerable difference in the amount of calcium taken up by eggs with or without jelly coats has been observed.

The experimental solution surrounding the eggs was sea water containing a small amount of radioactive calcium (maximum, 1.05×10^{-2} mg. or 0.52 μc per ml.). Uptake, by the eggs, was followed by measuring the disappearance of the isotope from the surrounding sea water. The experimental eggs were crowded (up to 3.5×10^6 per 10 cc.) in the bottom of a dish but constant agitation permitted them, when fertilized, to develop as quickly as dilute (10^3 per 10 cc.), non-agitated control eggs in non-radioactive, calcium-enriched sea water and in normal sea water (pH 8.2–8.4). There was no pH change in any dish and the temperature remained at 20.0° C. At least 98% fertilization was obtained in accepted experiments.

Eggs with jelly coats show an increase of Ca^{45} before and after fertilization followed by a decrease from the sixth to the tenth hour after fertilization, when hatching occurs. Eggs freed from jelly coats and washed repeatedly show no such increase and subsequent decrease. Eggs with jelly coats which have taken up Ca^{45} and then by gentle shaking gradually have their jelly coats removed, show a subsequent decrease of radioactivity until all the jelly coats are off. There is no measurable uptake of Ca^{45} by denuded eggs when the dissolved jelly coat material is left in the medium in which the eggs are suspended.

The simplest conclusion is that some substance in the jelly coat binds calcium as the jelly coat swells, and loses it preparatory to hatching. At present, however, it is impossible to state what proportion of the Ca^{45} is exchanged and what proportion is accumulated.

---

[1] Work done during tenure of Atomic Energy Commission Predoctoral Fellowships in the Biological Sciences.

The effect of tissue extracts on cell division. L. V. Heilbrunn, W. L. Wilson and D. Harding.[1]

The appearance of the mitotic spindle and the division of a cell are preceded by a gelation of the protoplasm, the mitotic gelation. In view of the fact that the gelation or clotting of protoplasm is typically a process similar to blood clotting, it is not surprising that the mucopolysaccharide heparin prevents mitotic gelation just as it prevents the clotting of vertebrate blood. This was shown to be true by Heilbrunn and Wilson (1949) and they also showed (1950) that the bacterial polysaccharide of Shear, which is either a heparin or a heparin-like substance, also prevents mitotic gelation and cell division. The bacterial polysaccharide can cause regression of tumors in mice, but it is toxic for men. In view of the fact that heparin-like substances are found in practically all types of living material, we felt that it might be possible to extract from some tissue a mucopolysaccharide which would cause regression of tumors in men as well as in mice.

After various unsuccessful attempts, we found that extracts made with acidified sea water were very effective in preventing the mitotic gelation as it occurs in the eggs of the worm Chaetopterus. These extracts were prepared from the posterior segments of the Chaetopterus, from the clam Mactra, from the ovaries of the sea urchin Arbacia and the sand dollar Echinarachnius; but by far the most potent extracts were obtained from the ovaries of the starfish Asterias. These extracts prevent the mitotic gelation and they also prevent cell division. They also cause a liquefaction of the cortex of the egg.

The extract from the starfish ovary is more potent if the ovary contains few rather than many eggs. The active substance in the extract is heat stable.

The action of heparin on fertilization in the eggs of Arbacia punctulata and Echinarachnius parma. Clifford V. Harding.[2]

Dilute solutions of jelly coat substance inhibit the normal differentiation of the fertilization membrane (*Exp. Cell Res.*, 1950). It became of interest to test the action of other substances, chemically related to the jelly coat substance, on fertilization. Three samples of heparin (Hynson, Westcott, Dunning; Upjohn; Vitrum) were tested for their action on fertilization and first cleavage (see also P.S.E.B.M., 1949, **70**). Hynson, Westcott, Dunning heparin proved to be the most active and in concentrations of 0.025 per cent effectively altered normal fertilization (the membranes remained very low and granular). Moreover, this heparin had the same effect on membrane formation in eggs which were activated with hypertonic sea water. This suggests that the heparin has its effect primarily on the egg and not the sperm. A concentration of 0.2 per cent heparin completely blocked fertilization in the eggs of Arbacia, and they did not divide. This prevention of cleavage is most likely due to a blocking of fertilization, since eggs placed in heparin after fertilization cleave normally (see A. Gagnon's abstract). Eggs which were treated with trypsin to remove the vitelline membrane, fertilized in sea water, and transferred to 0.2 per cent heparin in Ca-free sea water (to dissolve the hyaline layer) showed high percentages of cleavage. In this case, the external layers were removed to insure penetration of the heparin to the egg surface.

Heparin solutions treated with small amounts of sodium periodate did not prevent membrane elevation or cleavage. Heparin Vitrum and Upjohn heparin were relatively weak in their action on fertilization. However, if the solutions were autoclaved for 10 minutes at 250°F. they became active in preventing membrane elevation in concentrations of 0.025 per cent. Treatment with periodate removed this effect. Fucoidin, a polysaccharide, and the human H substance also inhibited membrane elevation. The activity of these was removed with periodate. These results are of interest in relation to the work of Runnström and Kriszat.

---

[1] Aided by a grant from the U. S. Public Health Service.

[2] National Institutes of Health post-doctorate fellow.

The action of heparin on fertilization and cleavage in some marine eggs. André Gagnon.[1]

Earlier work has shown that heparin tends to prevent both fertilization and cleavage.

The eggs of *Mactra solidissima*, *Arbacia punctulata*, and *Echinarachnius parma* were studied. A 0.2% heparin solution (Hynson, Wescott, and Dunning) made up in filtered sea water was used in all the experiments.

In one series of experiments, the eggs were placed into the heparin solution at various intervals before fertilization, then fertilized and allowed to develop in it. With Mactra eggs thus treated, there results a high inhibition of cleavage (as high as 92%). Treatment of Arbacia eggs under similar conditions gives even higher cleavage inhibition (average 95%). The results also indicate that the time of exposure of the eggs before fertilization does not affect the final high percentage of inhibition. With the Echinarachnius egg, preliminary work indicates that there is a delay, but that ultimately cleavage is almost the same as in the controls. In other experiments, the eggs were fertilized in sea water and then exposed to the heparin solution at various intervals after fertilization. The following results were obtained. With Mactra there is some inhibition, and the period of sensitivity is restricted to a short period following the addition of the sperm. In Arbacia and Echinarachnius, there is essentially no inhibition. From these results, it is concluded that the main step affected in Arbacia and Mactra is fertilization. In Arbacia, eggs that are fertilized in heparin lack a fertilization membrane (see C. Harding's abstract). In Chaetopterus, Heilbrunn and Wilson have found that sperm penetration and fertilization may occur in the presence of heparin; yet, there is inhibition of cleavage.

An experimental study of maturation in Chaetopterus eggs. Lester Goldstein.

Since eggs of *Chaetopterus pergamentaceus* apparently mature as a result of leaving the inhibiting environment which surrounds the ovary, and not by any stimulus provided by the sea water environment, an attempt was made to find what substance, or substances, was inhibiting maturation *in vivo*. Experiments utilizing sea water saturated with carbon dioxide showed that one of the inhibitors could very well be carbon dioxide. Carbon dioxide solutions would completely inhibit maturation and reversibility was also complete when the eggs were returned to normal sea water. Though carbon dioxide causes sea water to become acid, it was demonstrated, over a wide range of pHs, that the maturation process was not affected by any pH value in itself. Consequently, the inhibition is probably due to a specific action of carbon dioxide.

While carbon dioxide, which may accumulate in the ovaries due to the respiration of the eggs, may be an inhibitor, it probably can account for only about 50 per cent of the inhibition within the animal. Another inhibiting factor may exist *in vivo* and preliminary experiments indicate that this is possibly a protein or a compound bound to a protein.

Other experiments, utilizing sodium citrate, show that calcium ions are necessary for maturation to proceed. This calcium is necessary in or on the eggs, since maturation will proceed normally in environments lacking calcium.

Changes in the protoplasmic cortex during cell division. Walter L. Wilson.[1]

During the course of cell division, changes occur in the colloidal state of the cell cortex. In the egg of the annelid worm, *Chaetopterus pergamentaceus*, it is possible to make a physical study of the protoplasmic cortex. For in this egg, granules can move from the cortex under moderate centrifugal force, and the force necessary to dislodge the granules varies during the mitotic cycle.

In the present work, eggs were subjected to various centrifugal forces at intervals between fertilization and cleavage and the cell cortex examined under high power magnification to determine whether or not the granules were present in the cortex. Shortly after fertilization (at 21.0°C.) the rigidity of the cortex decreases. After several minutes the rigidity of the cortex increases again. At about 10 minutes after fertilization the cortex has returned to its original rigid condition and remains so until about 35 minutes after fertilization, at which time there is a decrease in the cortical rigidity. The cortex remains in this condition until the cell divides. Thus the cell cortex becomes rigid prior to the gelation which is known to occur in the cell interior. Experiments carried out while the cleavage furrow was forming show that there is no increase in the rigidity of the cortex at this time.

---

[1] Aided by a grant from the U. S. Public Health Service.

Microscopy in the ultraviolet visible and infrared. GEORGE I. LAVIN.

In 1946 a description of a set-up (*Science,* 104: 61) was published whereby photomicrographs, using various wavelengths of light, could be obtained. It was found that this arrangement was rather cumbersome in use. In the present ensemble the microscope and camera are placed on the top of a small table. The quartz mercury resonance lamp is mounted directly underneath the table top. The light from the lamp reaches the microscope through a hole bored through the table.

With this source and a cobalt sulfate nickel sulfate filter the 2537A° mercury line can be isolated and used to image and focus a field with a willemite screen. The 5460A° line, from the same source, can be obtained with a Wratten 77A filter. This can be focussed with a ground glass screen. The light from a tungsten lamp is introduced by means of a right angle prism, which can be thrown in and out of the field. A Wratten 25A filter is used to obtain a field and focus after which an 88A filter is used in conjunction with infrared sensitive plates.

Preliminary results indicate that different structures are revealed by use of the various regions of the spectrum. The method has been applied to a study of Arbacia eggs and plutei.

Invertebrate photoreceptors. LORUS J. MILNE AND MARGERY J. MILNE.

A review of the extensive and widely scattered literature on light perception among invertebrate animals has been made to furnish a more integrated basis for future research in this field, along both anatomical and physiological lines. The range of photosensory mechansims is far more impressive than that to be found among the vertebrate series, and analogous mechanisms appear to have arisen independently in very many places. Perhaps the most distinctive feature of the survey has been the widespread and important reliance of invertebrates upon dermal light sensitivity without distinctive sensory structures, and their dependence upon such systems for interpretations of presence or absence of light, changes of intensity (whether sudden illumination or shadowing), of direction of light source, or spectral content. Often behavior studies have indicated far more excellence in light perception than would be predicted from structural investigations. Correspondingly, the supplementary action of dermal sensitivity in many instances renders difficult the interpretation of vision by more highly complex photosensory structures. Among the polychaete annelids, the gastropods, pelecypods and cephalopods, and certain arachnids, useful image formation appears to be provided for—often with mechanisms allowing accommodation as well as the more widespread adjustments in sensitivity with dark or light adaptation. In a few polychaetes, gastropods, and crustaceans and insects, compound eyes furnish the basis for mosaic vision with varying degrees of resolution and sensitivity. Embryological information and details of degeneration of visual mechanisms in parasitic, abyssal, speleologic and fossorial species have provided further understanding of the inter-relationships between functional photoreceptors.

The loci of action of ultraviolet and x-radiation, and of photorecovery, in the egg and sperm of Arbacia. HAROLD F. BLUM, J. COURTLAND ROBINSON AND GORDON M. LOOS.

Delay of cleavage of Arbacia eggs by ultraviolet radiation (λ 0.27μ to 0.313μ), is associated with the nucleus: The cleavage of enucleate halves is not delayed if these are irradiated before fertilization. But if the sperm nucleus is introduced by fertilizing enucleate halves before irradiation, or if the sperm itself is irradiated before fertilization, delay occurs. Nucleate halves, like whole eggs, show delay of cleavage under any of the above conditions. Thus, only when a nucleus (of either egg or sperm) is exposed to ultraviolet radiation does delay result.

Photorecovery after exposure to ultraviolet radiation is associated with the cytoplasm of the egg: Illumination of sperm with visible radiation ($\lambda \backsim 0.4\mu$ to 0.5μ) after exposure to ultraviolet, does not diminish the delay of cleavage of eggs fertilized with this sperm. If eggs are illuminated after fertilization with ultraviolet irradiated sperm, photorecovery is observed. This is also true for nucleate and enucleate halves when treated in the same way. Photorecovery occurs in whole eggs, nucleate, or enucleate halves when fertilized with normal sperm and then exposed to ultraviolet radiation. When eggs or nucleate halves have received ultraviolet radiation before fertilization with normal sperm, photorecovery is observed. Under such conditions, photorecovery cannot be demonstrated in enucleate halves, there being no delay of cleavage. Thus, photorecovery occurs in whole eggs, nucleate or enucleate halves, but not in sperm. Sperm and egg are comparable here to bacteriophage and *E. coli* in Dulbecco's experiments.

Similar experiments with x-radiation relate the locus of cleavage delay to the nucleus. No photorecovery was demonstrated in the experiments with x-rays, suggesting a fundamental difference in mechanism of action from that of ultraviolet radiation.

Integumentary pigmentation and the coelomic fluid of Thyone Briareus (Lesueur).

NORMAN MILLOTT.

The black color of Thyone is due to an integumentary pigment occurring either as free granules or within cell bodies, some of which resemble degenerating amoebocytes of the type found in the coelomic fluid.

The pigment shows the chemical properties characteristic of melanin (Lison, 1936). The participation of the amoebocytes of the coelomic fluid in pigment formation is at once suggested by the fact that when the coelomic fluid is exposed to air it throws down a clot, including many amoebocytes, which slowly changes from white through yellow to brown, and eventually breaks up into irregular globular masses. These strongly resemble the black bodies found normally occurring in the perivisceral coelom. The depth of color of the clot can be increased by addition of a trace of catechol to the coelomic fluid *in vitro*. Coelomic fluid *in vitro* will oxidize other phenols such as "dopa," pyrogallol and p-amino-phenol at pH 7.4, 7.6 and 30°C. Only a few samples oxidised guaiacol, and tyrosine is slowly oxidised at pH 7.0 and 30°C. only when a trace of catechol is added to the substrate. These actions are slow and feeble, requiring 9–20 hours in order to produce a distinct color reaction. They are abolished by boiling the coelomic fluid or adding 0.0005 M–0.001 M cyanide. Whether these oxidations are truly enzymatic must remain uncertain. The coelomic fluid shows a well marked positive oxidase reaction with α-napthol and paraphenylene diamine (Snell and Snell, 1937), but it is not attributable to enzymes, being unaffected by 0.001 M cyanide and by boiling for 30 minutes. Possibly the action may be due to substances such as orthoquinones (Pinhey, 1930; Wheldale-Onslow, 1931).

No indication has yet been obtained of the substrate on which these substances may act *in vivo,* but it is significant that spectophotometric examination of the centrifuged coelomic fluid revealed no tyrosine in either amoebocytes or the fluid itself. Similarly, no color reaction was given with Millon's and Morner's reagents, although a feeble positive reaction was shown with Folin and Denis' reagent.

The feeble oxidation of phenols cannot be due to a low oxidation-reduction potential, for E_0' for the coelomic fluid in equilibrium with air at pH 7.0, estimated colorimetrically, is +0.218– +0.224. Similarly there is no evidence of an interfering dehydrogenase, for methyl alcohol does not increase the rate and extent of darkening of the coagulum in the coelomic fluid *in vitro,* nor do dehydrogenase inhibitors such as acetone, chloroform and phenyl urethane appreciably affect the reaction with α-napthol and paraphenylene diamine.

In order to ascertain whether amoebocytes carrying effete substances will deposit them in the pigment bearing areas, iron saccharate in sea water was injected into the perivisceral coelom. Amoebocytes were observed to take up the saccharate, some of which was eliminated via the cloaca, but further details must await the preparation of sections. It may be mentioned that methylene blue injected into the perivisceral coelom in connection with oxidation-reduction studies, was seen to be eliminated rapidly by the gut, respiratory trees and bodywall. Both active transfer by the gut wall and the activities of amoebocytes are involved, the latter, on occasion, being so active in elimination that the coelomic fluid becomes almost devoid of them. That many amoebocytes are lost via the cloaca may be shown by inserting a plug, when it is seen that not only a large measure of dye but also many of the amoebocytes are retained in the coelomic fluid. It is noteworthy that the amoebocytes tend to deposit granules of dye in those areas of the respiratory trees already bearing accumulations of black and brown pigment; and further, that the dye often appeared red in these areas, owing to metachromasy.

Micro-injection of $CaCl_2$ into the giant nerve axon of the squid. ROBERT CHAMBERS AND CHIEN-YUAN KAO.

In micro-operations, it has been general experience that inorganic salts of common monovalent cations liquefy, while those of divalent cations coagulate protoplasm. The cells which show these phenomena are frog muscle fiber, sundry Echinoderm eggs, protoplast of plant cells, (root hairs of Limnobium), and protozoa (amoeba and Stentor). Recently, Hodgkin and Katz (*J. Exp. Biol.*, 1949) reported that the axon material of the giant nerve of the Squid reacts in a different way from that stated above. We have confirmed their results, although not agreeing with the claim of their reference on Stentor. In our experiments we used long tapering micropipets with diameters 30 micra across at 600 micra from the tip which measured 4 micra across. The micropipets were thrust diagonally into the interior of the axon with minimal injury indicated by the maintenance of a colorimetric pH of ca. 6.8 and by the ovoid shapes assumed by oil drops deposited within. Injected solutions of NaCl, KCl and water caused no change whereas, with divalent cations, the oil drops rounded up indicating a fluid environment. The effects were almost immediate for Ca and Sr, and delayed for Mg and Ba. The oil drops appeared always coated with a structureless film which prevented union of the drops when in contact. This film was more evident before than after injection of $CaCl_2$. Sodium alizarin sulfonate, introduced after $CaCl_2$, caused a precipitate whereupon the axon material again gelated but the oil drops were distorted irregularly indicating that the jelly had lost the linear arrangement characteristic of the normal fiber.

Some low frequency characteristics of axoplasm and the nerve membrane. OTTO H. SCHMITT.

Measurements of membrane characteristics by the complex attenuation method in the range 40–10,000 c.p.s. on normal squid nerve emphasize the existence of at least three separate electrical components, one relatively stable and probably associated with physical structure, a second directly related to ionic permeability, and a third which is highly responsive to physiological state. The stable component is responsible for the familiar 1 mfd./$cm.^2$ capacitance and has a relatively small temperature coefficient. The permeability term is closely associated with excitability but has an unpredicted frequency minimum. The unstable susceptance component is highly temperature sensitive and responds sharply to ionic changes in the environment.

The volume resistivity of squid axoplasm in the intact axon has been studied as a function of temperature, frequency, and fiber size by a method which permits correction for the conductivity of solution clinging to the outside of the nerve. The mean conductivity value found is 22.5 millimhos cm. at 20°C. or about 51% of the conductivity of Woods Hole sea water. Conductivity changes almost precisely as does that of appropriately diluted sea water in the range 5° to 30°C. No detectable variation of resistivity with frequency is found above 500 c.p.s. and a slight measured variation in the 100 c.p.s. region is probably a by-product of the rapidly changing membrane impedance in this frequency range. No systematic variation of axoplasmic conductivity with fiber size has been noted.

Progressive changes in the nerve membrane admittance after dissection. PETER A. STEWART.

The time changes in the membrane conductance and susceptance of the squid giant axon have been determined by the complex attenuation method in order to establish the normal process of deterioration in an isolated nerve fiber. It is found that the electrical state of the membrane remains almost constant for two or three hours if the nerve is frequently bathed with sea water. This steady state is characterized by full excitability, constant susceptance, and a constant membrane conductance of about 1 millimho/$cm.^2$ (at 100 c.p.s.). After two or three hours, the conductance begins to rise, increasing linearly with time for several hours. Excitability is lost when the conductance reaches about 4 millimhos/cm.^{2}

If the nerve is not bathed, the conductance rises continuously, no steady condition is observed, and excitability is again lost at a conductance of about 4 millimhos/cm.^{2} In both these cases, the susceptance changes relatively little, although a gradual decrease with time is observed at frequencies above 500 c.p.s.

All these changes seem to be completely irreversible and inescapable. Changes produced by experimental agents such as temperature or chemicals are superimposed on this general pattern.

Washing the nerve with potassium-free saline delays the rise in conductance and loss of excitability by an hour or two, but does not change the general pattern. The effect of washing with a large excess of potassium (isotonic KCl) is to eliminate excitability instantaneously and to raise the conductance by a factor of three or more within a few minutes. After this the membrane deteriorates rapidly and the conductance increases to a very high value as the membrane disappears.

Electrically induced optical and dimensional changes in single axons including the squid. Preliminary observations on ion effects. JULIAN M. TOBIAS.[1]

Insofar as the longitudinally oriented e.m.f. impressed upon an axon by the propagating nerve impulse is mimicked by an electrode-applied, longitudinally oriented e.m.f., it is useful to examine changes (electrical, chemical, structural) under and around such polarizing electrodes in an attempt to anticipate what changes may occur at the similarly polarized regions of the propagating impulse.

Platinum electrode polarization of whole nerves or single axon of crayfish, spider crab, *Carcinus maenas,* lobster or frog produces reversible cathodal swelling with increased transparency and increased stickiness whereas anodally one sees shrinkage with increased opacity and increased rigidity. In addition, the single frog axon, but not the others, shows a quick, reversible and fatiguable anodal shortening and subsequent lengthening and marked surface wrinkling on closing and opening the circuit.

These changes are, however, apparently due in part to local electrochemical changes unique for polarizable electrodes (probably pH), since with non-polarizable electrodes (Ag-AgCl or balanced salt wicks leading to calomel half cells) one sees neither the quick axonal "twitch" nor the surface wrinkling in the frog axon, nor the rigidity or adherency changes in any of the axons examined.

With non-polarizable wick electrodes changes are most clearly seen in squid (*Loligo pealii*) giant fibers. There is anodal shrinkage and frequent plasmolysis of the protoplast inside the sheath with marked clouding of the axon. Cathodally there is swelling with excessive transparency. The cloudy material can be moved within the axon by pressure, and when given an opportunity it migrates toward the cathode. These changes are reversible on reversing polarity. Birefringence changes characteristically at the two electrodes. If, however, the axon be polarized with a small pool of isotonic KCl around the anodal wick and a small pool of isotonic $CaCl_2$ or $MgCl_2$ around the cathodal wick then the anode produces increased transparency, the cathode increased opacity.

Preliminary observations on squid axon structure. Light scattering properties using an intracellular light source and mechanical prod. SIDNEY SOLOMON AND JULIAN M. TOBIAS.[1]

To investigate axonal ultrastructure, a method studying light scattering by cellular structural elements (molecular and macromolecular) as a function of activity is being developed. A quartz microneedle, impaling the axon along its long axis, serves both as a mechanical prod and as an intracellular light conduit. So far only white light and magnifications up to 100 × have been used. Only the squid axon has been examined.

---

[1] This work has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the Universtiy of Chicago.

Three observations of interest have been made: (1) Light emerging from the quartz needle tip and propagated along the long axis of the axon is scattered to produce a typical Tyndall effect. At the magnifications used (100 ×) no otherwise visible granules have been resolved (except as below). This is compatible with the notion that the axoplasm is a colloidal system. (2) More vigorous movement of the prod in the axoplasm produces a local increase in fluidity and the appearance of visible granules. This structural alteration appears to be reversible on standing, but only during the early phases of an experiment. (3) When the prod is slowly and evenly advanced in the axoplasm, a series of alternate, stationary, light and dark, curved bands (concave toward the prod tip) is seen to form in the axoplasm ahead of the tip. These appear to be cup-shaped about the tip and do not seem to extend laterally to include the sheath. Lateral extent may, however, be a function of prod size. Although band spacings and curvatures have been measured and an attempt has been made to correlate these dimensions with the prod tip size and shape, the geometry of the system has not otherwise been controlled.

It is hoped that further investigation will extend and clarify these observations.

Renal chloride excretion by the smooth dogfish, Mustelus canis. RUDOLF T. KEMPTON AND LEONORA BERMAN.

In 51 collection periods on 12 large female smooth dogfish, inulin has been used as a measure of glomerular filtration, and the reabsorption of water, chloride and urea examined simultaneously. Data on the total molecular concentrations as reflected by the depression of the freezing point have not yet been studied.

While the reabsorption of urea from each ml. of filtrate is related to blood level in such a way that a constant concentration of urea is left in the tubule, no such relationship appears with chloride. Instead the reabsorption of chloride from each ml. of filtrate is approximately constant, averaging 10.4 mgm. (expressed as NaCl), with 66% of the values falling between 8.4 and 12.4. Variations are not related to any factor or condition yet uncovered from the data. Water, urea and chloride reabsorption are entirely independent of one another, and there is no evidence of an osmotic control of either urea or chloride reabsorption.

Total chloride reabsorption (mgm./hr./k.) is related to both the volume of filtrate and the amount of chloride filtered. Since the blood level is quite constant, variations in these two factors are similar. Variations in the volume of filtrate appear to be due to varying numbers of active glomeruli, since the chloride reabsorbed from each ml. of filtrate is unaffected by a range of filtration rates in which the maximum is over seven times the minimum. The data suggest that there is a maximum rate of chloride reabsorption somewhere between 30–40 mgm./hr./k. (as NaCl). Artificially raised blood chloride levels will be necessary to establish this point.

Consideration of the U/P ratios leads to the conclusion that relatively minor differences in reabsorption may result in a change from urine in which the concentration of chloride is below blood level to urine in which the chloride is much more concentrated than the blood. Variations in water reabsorption have little determining effect on the U/P ratios. Since an active reabsorption must be invoked to explain the U/P ratio that is below 1.0, the continuity of the data seems to demonstrate that an active reabsorptive mechanism is functioning even when the concentrations are such that the reabsorption is in the direction of a gradient which would permit passive outward diffusion.

Observations on the flight motor of Diptera. EDWARD G. BOETTIGER AND EDWIN FURSHPAN.

Electrical activity not associated with wing movement was detected in the thorax of all (eight) species of Diptera tested, when treated with ether, carbon tetrachloride, and certain other agents to the point of loss of normal reflex activity. These potentials, at a certain depth of anesthesia, are very similar to flight potentials. They are only found in sections of the thorax containing the thoracic ganglion, and simpler, more regular discharges, consisting of a small "end-plate-like" potential from which a full spike grows, may be recorded from the ganglion enclosed in a minimal amount of muscle tissue.

Carbon tetrachloride, in addition to producing large ganglionic potentials, causes contraction of the muscles and small, regular potentials in the absence of the ganglion. Whole flies under carbon tetrachloride show anesthetic flight and general contraction of the thoracic musculature, which results eventually in cessation of flight due to the wings' being caught in the extreme up or down position (usually up). Then on depressing the posterior end of the scutellum, flight may recur or the wings may "click" into the down position. Dissection of flies under carbon tetrachloride reveals a sudden change in the length of the muscles on passing the clicking point. The path thus travelled by the wings may be strikingly similar to that of normal flight, including the characteristic changes in the angle of attack.

It is suggested that the flight motor, capable of 60,000 cycles per minute in certain midges, depends upon a constant tension-producing excitation of both direct and indirect muscles, initiated by the ganglionic potentials; and that the click phenomenon, resulting from the application of this tension to the peculiar wing articulation, allows a sudden change in the loading of the indirect muscles during flight so as to quick-release the muscles causing the particular movement, and to quick-stretch its antagonist. Resulting tension changes then favor the opposite movement.

Dependence of ciliary action upon acetylcholinesterase activity. GERALD R. SEAMAN.

The ciliate *Tetrahymena geleii* contains an active acetylcholinesterase. The enzyme splits 0.08 μg acetylcholine/hr./mg. dry weight of tissue. The enzyme does not attach butyrlcholine. Eserine (4×10^{-7}M) and DFP (4×10^{-7}M) inhibit hydrolysis of acetylcholine by the enzyme preparation from *T. geleii.* The inhibitors, at concentrations of 3.85×10^{-3}M and higher, inhibit motility of the organism. DFP and eserine apparently inhibit specifically acetylcholinesterase and not energy supplying enzymatic reactions; cells immobilized by inhibitor treatment have the same glycolytic rate and the same rate of oxidation of carbohydrate as do untreated cells. The inhibitions of motility effected by DFP and by eserine are completely reversible by washing.

DFP and eserine also inhibit ciliary activity of the frog esophagus and of the cill of *Venus mercenaria.* The inhibitions in these cases are also completely reversible by washing.

It is concluded that the fibrillar system connecting the base of individual cilium is actually conducting tissue and as such is dependent upon acetylcholinesterase activity.

Microphallid metacercariae encysted in Limulus polyphemus. HORACE W. STUNKARD.

In the summer of 1950, Dr. T. H. Waterman found metacercariae encysted in the eyes of *L. polyphemus.* Examination of a large number of horseshoe crabs disclosed that all of them were infected and larvae were found in the connective tissue of the liver and other organs as well as in the eyes. The metacercariae contained well developed gonads and the seminal receptacles were filled with active spermatozoa. They were fed to baby chicks, half-grown white mice, and young golden hamsters. Excysted larvae were found in the intestine of a white mouse 12 hours after it was fed. Worms recovered from a mouse 4 days after feeding, contained eggs in the uterus. They proved to be microphallid trematodes, similar to and possibly identical with those described by Cable and Hunninen (*Biol. Bull.*, 1940, **78**: 136) as *Spelotrema nicolli.*

Larval trematodes from the planarian, Dugesia tigrinum. HORACE W. STUNKARD.

During the winter of 1949–1950, Mr. J. Louis Bouchard of the Oklahoma A. and M. College found larval trematodes in specimens of *D. tigrinum* obtained from the Marine Biological Laboratory. The worms were referred to me for possible identification. The structure of the larvae indicated that they were azygiids, but no further information was available. Records of the Supply Department of the M. B. L. showed that the planarians were collected from Morse's Pond. Planarians collected from this pond in August, 1950 contained larval worms of the same species and search was made for the snail host of the parasite. Small snails, *Amnicola limosa,* were found infected with the asexual stages of the trematode. Planarians, examined under the microscope and known to be uninfected, were placed in a finger bowl with specimens of *A. limosa* which were shedding the cysticercous cercariae. After several days exposure, the larval trematodes were found in the pharyngeal pockets of the host, as many as four in a single planarian. The larvae do not encyst or grow while in the planarian, which obviously serves merely as a transfer host. Planarians infected with the larvae have been fed to various fishes.

Notes on growth and cystment in Dileptus binucleatus. ANNA JOSEPHINE BRIDGMAN.

From soil and débris collected in a temporary pool near Chapel Hill, N. C., in 1944, animals identified as *Dileptus binucleatus* have been excysted from time to time. It is only necessary to add tap water to the soil in a finger bowl to find, within twenty-four hours, numbers of these ciliates swimming about.

It was found, after some experimentation, that they could be cultured in a modification of Loefer's culture medium with the addition of generous numbers of Halteria.

Prowazek (1904) briefly described the encystment of Dileptus, attributing it to cold. Studitsky (1930) says that in *Dileptus gigas* it is due to increased salt concentration. Experiments indicate that in *D. binucleatus* it may also be due to decreased salt concentration, since they encyst in distilled water, to lack of food, and perhaps to other unfavorable factors.

The cysts are approximately 60 μ in diameter, have one vacuole, and are slightly yellowish. There are three membranes: an outer clear gelatinous covering which serves to secure them to some solid object; a thick striated mesocyst, apparently non-elastic; and an inner, elastic endocyst.

The excystment process is not essentially different from that described for other ciliates. If the culture fluid in which the cysts are formed is removed and replaced by distilled water or fresh culture fluid the animals will excyst. The beginning of the process is marked by an increase in the size and number of vacuoles. As they increase the animal differentiates, there is considerable movement within the cyst, and the ecto- and mesocysts rupture. The animal, still within the endocyst, bulges out from the original cyst wall, but does not escape. The inner membrane is elastic and stretches considerably at this time, while the outer membranes, still enclosing part of the animal, seem to give very little. The cilia, now well developed, enable the animal to move actively within the cyst, pushing against the endocyst. This squirming may go on for fifteen or twenty minutes. Eventually there is a relatively small break in the endocyst and the animal squeezes through, with the proboscis leading and the rear half of the body somewhat flattened and folded on itself. It swims slowly at first, but soon assumes its usual shape and activity.

The excystment process is usually accomplished in less than two hours, although it may extend over several. The shortest observed time is one hour and twenty minutes.

The position of Japanese varieties of Paramecium caudatum with respect to American varieties. LAUREN C. GILMAN.

The occurrence of ten varieties of two matings each in collections of *Paramecium caudatum* from various localities in the United States has been previously reported (*Biol. Bull.*, 97:239). In addition an eleventh variety which gives no reaction with the previous ten was found in collections made in New Haven, Connecticut in the summer of 1949. This variety was designated as variety 11 and the mating types present as XXI and XXII in accordance with the scheme used on the other varieties.

The presence of four non-interbreeding varieties of *P. caudatum* in collections from the Island of Honshu was reported by Hiwatashi (*Sci. Repts. of the Tohoku U.* 4sr. (Biol.) **18**: 137–140). Representative cultures of these four varieties were obtained from Hiwatashi in Sept., 1949 through the kind cooperation of the Medical Department of the Occupation Forces. Each variety consists of two interbreeding mating types as do the American varieties and were designated according to the same system.

Samples of each of the Japanese varieties were mixed with samples of each of the American varieties when, as the result of test mixtures with appropriate controls, all were known to be in good reactive condition. The mixtures were examined immediately for mating reactions and later for conjugation.

The results of the mixtures are shown below. Corresponding mating types in the two systems are found on corresponding lines. Where no corresponding variety was present in the American collections a new designation according to the American system was given.

Gilman's variety	System type	Hiwatashi's system	
		Variety	Type
1	I	1	1
	II		2
2	III		
	IV		
3	V	3	6
	VI		5
4	VII		
	VIII		
5	IX		
	X		
6	XI		
	XII		
7	XIII		
	XIV		
8	XV		
	XVI		
9	XVII		
	XVIII		
10	—		
	XX		
11	XXI		
	XXII		
12	XXIII	2	3
	XXIV		4
13	XXV	4	7
	XXVI		8

There are now known, then, thirteen varieties in *Paramecium caudatum*. Two of these, variety 1 and variety 3, are found in both the United States and Japan. Nine, varieties 2, 4, 5, 6, 7, 8, 9, 10, and 11 are so far found only in the United States. Two, varieties 12 and 13, are known so far only from Japan.

The marine sand dwelling Ciliates of Cape Cod shores. E. Fauré-Fremiet.

Two categories of Ciliates species dwell in the damp sand of marine beaches; some of these seem to be so closely adapted to interstitial conditions that they do not live outside; some others enter the sand easily and can live in it as well as anywhere else along the seashore, where they are ordinarily widespread.

With regard to the size of the interstitial sand spaces, which are roughly proportional to the size of the grains, the sand dwelling Ciliates can be divided into two ecological classes: In the coarse sand, with a mean diameter of the grains above 0.4 mm., the "mesoporal" Infusorian fauna includes few species exclusively sand living, and many occasional sand dwelling ones. In fine sand, with a mean grain diameter of 0.3 to 0.7 mm., the "microporal" Infusorian fauna is composed of true sand-living Ciliates species. In addition, some typical interstitial species, widespread into fine and coarse sands, may be called "euryporal."

Comparison of the Ciliates interstitial fauna observed in Brittany and Cape Cod beaches shows that interstitial species are cosmopolitan and that the distinguishing characteristics of meso- and microporal fauna have a generalized ecological significance. On the other hand, it appears that the truly microporal species are endowed with some adaptive peculiarities very interesting from the standpoint of cytology, cell-physiology and micro-ecology.

Presence of Protohydra leukarti Greef at Barnstable Harbor. E. FAURÉ-FREMIET.

No abstract submitted.

Vascular patterns and behavior in typical parapodia of Nereis virens and Nereis limbata. PAUL A. NICOLL.[1]

Although activity of vascular systems is usually associated with mechanisms that circulate the blood or regulate pressure gradients, many observations indicate blood vessels may exhibit motor phenomena that serve some other purpose.

The remarkable alternation of contraction and relaxation, in a more or less rhythmical pattern, exhibited by arterioles and precapillary sphincters in many tissues of vertebrates, and by the veins in a few cases such as the wing membrane of bats, has been termed rhythmical vasomotion. This activity certainly is not directly involved in circulation of the blood, nor does it appear to modify any total pressure gradients within the system. It is rather an independent effector mechanism mediated by activity of individual muscle cells in the vessel wall, and in most instances is not primarily under control of the nervous system.

It was hoped that the great diversity of form of the vascular systems in the Polychaetes would make them a profitable group to study the nature of the behavior of such small vessels. However, it soon became evident that detailed studies on the vascular systems of the available Polychaetes were lacking. After a preliminary survey, work was concentrated on the parapodial circulation of *Nereis virens* and *Nereis limbata.*

The general characteristics of the vascular systems of the two forms are very similar. The classical description for *N. virens* by Turnbull, which has been followed by recent authors, is both incorrect and inadequate in many details. No segmentally arranged direct connections occur between the dorsal and ventral longitudinal vessels. Rather, three lateral segmental vessels are present that are functionally quite separate. One arises from the dorsal vessel; a second originates, at least functionally, from the intestinal capillary plexus; and the third arises from the ventral vessel. In *N. limbata* this third vessel has two important branches.

Space does not permit a description or discussion of these vessels or their activities. Each makes some anastomoses through the superficial capillary plexus with the others which allows some circulation through the parapoda. More striking, however, is the fact that to a large but variable extent, each vessel terminates in a rich arborization of blind-ending tubes. The high contractility of these blind tubes and their supplying vessels results chiefly in an ebb and flow of blood, rather than true circulation, that must produce local pressure changes similar to those developed in capillaries of vertebrates by rhythmical vasomotion.

Failure to find intercellular protoplasmic continuity in Griffithsia globulifera. HOWARD J. CURTIS AND JOHN L. MYERS.

It has been claimed by many investigators that there is protoplasmic continuity between adjacent cells in many plants, and the red marine alga, *Griffithsia globulifera,* is said to exhibit

---

[1] Supported in part by a U. S. Public Health grant.

this very conspicuously. Evidence for this concept has been almost entirely of a morphological nature, and the present work is an attempt to add evidence of a physiological nature. Strands of single cells of Griffithsia were isolated and placed in a recording chamber consisting of two electrode wells cut in a block of wax. The wells were filled with isotonic KCl or sea water with the cell-strand bridging the space between the wells, and the whole chamber was covered with a layer of mineral oil. There were usually about three cell-junctions between the wells. With KCl in one well and sea water in the other a substantial resting potential was observed only if a substantial fraction of one cell protruded from the KCl. As the strand was pushed over so that more of the cell was in KCl, the potential fell and became practically zero as the meniscus approached the cell junction. On pushing still further, the meniscus passed the junction so the KCl wet the next cell, and the potential increased again. The same results were obtained when both wells were filled with sea water and one end of a cell was mechanically injured. When several cells in a line were injured, the injury potentials would add to give a potential which was the sum of the individual injury potentials. The only reasonable explanation of these results would seem to be that there is a very high electrical resistance between the cytoplasm of adjacent cells, and thus, no protoplasmic continuity.

On the absence of a fine internal network in erythrocytes of Elasmobranchs. A. K. PARPART.

Erythrocytes from individuals of several species of Elasmobranchs (smooth dogfish, dusky shark, sting ray) have been examined by means of televised microscopy. The wavelengths of the light used for this study lay between 365 mμ and 420 mμ.

The red cells of blood freshly removed from these Elasmobranchs contain between 30 and 100 granules which are in active Brownian movement throughout the cytoplasm. These granules vary in size from 0.1 to 0.4 μ. The rapid movement of the granules throughout the cytoplasm, their continual collision with the cell and nuclear surfaces strongly suggest that not only is there an absence of a fine internal network in these red cells but that the hemoglobin in their interior is in true solution.

These granules in the red cells are probably the same type that Nittis and also Dawson brought out by vital staining.

The granules disappear from the red cells of blood that is allowed to stand at ca. 23°C. for 20 to 24 hours; but not if the blood is kept at 5°C.; nor if the cells are suspended in isotonic Na Cl and kept at 23°C. This suggests that the granules may be metabolites which are utilized by the cell and not replaced when the cell is kept *in vitro* in its plasma at 23°C. The granules are present in red cells that have been osmotically lysed, and show even more active Brownian motion. Dogfish red cells washed centrifugally three times with 0.33 M Na_2 SO_4 undergo a loss of the nucleoprotein from their nuclei without loss of hemoglobin from their cytoplasm. Cells thus treated show no loss of granules, though the Brownian movement of the latter becomes less marked, owing, probably, to increased viscosity of the cytoplasm.

The role of epidermis in amphibian regeneration as revealed by triploid and haploid transplants to diploid limbs. ELIZABETH DEXTER HAY.

The rolé of the wound epithelium in the regeneration of amphibian limbs has been a controversial subject. Rose has suggested that the epidermis dedifferentiates to give cells for the early blastema. The following experiment was designed to test this hypothesis.

Heteroploid epidermal transplants were used as a means of tracing the individual epithelial cells. Haploid, diploid and triploid cells were identified by the number of nucleoli in the nucleus and by nuclear size.

Pieces of tailskin removed from triploid *Rana pipiens* tadpoles were transplanted to the thighs of stripped hindlimbs of diploid tadpoles. Tailskin transplants from haploid tadpoles were placed on diploid and triploid host limbs. Such transplants are almost entirely epithelium and have a very thick basement membrane. The opposite limbs were stripped as controls. Both limbs of each animal were amputated through the ankle at this time and the regenerates were fixed at various stages of growth. Histological sections were stained with Heidenhain's hematoxylin. This paper reports the positive findings up to this time.

The transplanted epidermis migrates as a sheet to the tip of the limb and covers the wound surface. The transplant's basement membrane remains on the thigh in the region where the strip of tailskin was placed. This was followed by means of haploid epidermal transplants identified by nuclear size. Both haploid and triploid epidermis piles up at the tip of the limb 5 days after amputation. At 6 days after amputation this epidermal cap disappears. It is not possible to follow the haploid any farther because cells cannot be identified by nuclear size once inside the blastema due to the tremendous range in cellular size there. However, triploid cells can be identified by the number of nucleoli. Diploid blastema cells never have three nucleoli. Numerous triploid and tetraploid mesenchymal-like cells have been found inside the blastema of a 6-day regenerating diploid limb which had received a triploid-tetraploid transplant. By present histological criteria these epidermal cells inside the blastema have dedifferentiated. We are particularly interested now in determining whether these undifferentiated blastema cells which have come from the epidermis redifferentiate into the adult tissues of the new limb.

Transphosphorylation in annelid muscle. WARREN H. YUDKIN.[1]

Arginine phosphate, present in the molluscs, arthropods, and to a certain extent in echinoderms, is replaced in the annelids by two as yet unidentified compounds with the chemical characteristics of phosphagens. One of these phosphagens found in annelids behaves on hydrolysis very much like creatine phosphate, and so it was believed, provisionally, to be identical with creatine phosphate by Baldwin and Yudkin (*Proc. Roy. Soc. London B*, 1950, **136**:614). In the present investigation this hypothesis was tested by determining the ability of crude extracts of body wall muscle of *Glycera dibranchiata* and *Neanthes virens* to phosphorylate creatine using adenosine triphosphate as a phosphate donor. Previously, Baldwin and Yudkin showed that *G. dibranchiata* contains only a creatine phosphate-like phosphagen and that *N. virens* contains both an arginine phosphate-like and a creatine phosphate-like phosphagen. Ochoa (*Biochem. J.*, 1938, **32**: 237), Lehmann (*Biochem. Ztschr.*, 1935, **281**: 271), Lohmann (*Biochem. Ztschr.*, 1936, **286**: 28), and Baldwin and Needham (*Proc. Roy. Soc. London B*, 1937, **122**: 197) have provided some evidence that the phosphokinases which are responsible for the transference of phosphate from adenosine triphosphate to arginine or creatine are specific for their respective guanidine bases.

The test system used in the present experiments contained adenosine triphosphate, creatine or arginine as phosphate acceptor, $MgCl_2$, veronal buffer at pH 7.85 and a KCl extract of muscle which had been dialyzed against KCl solution for 48 hours. In some experiments NaF was added or $MgCl_2$ was omitted. Determinations of acid-soluble phosphate were carried out after the mixtures had been allowed to incubate for 45 minutes at room temperature. Under these conditions it was readily demonstrated that frog (*Rana pipiens*) muscle phosphorylated creatine but not arginine and that scallop (*Pecten irradians*) muscle phosphorylated arginine but not creatine. Similarly prepared extracts from *G. dibranchiata* and *N. virens*, on the other hand, esterified neither creatine nor arginine. It is unlikely, therefore, that the creatine phosphate-like phosphagen present in these forms is identical with creatine phosphate.

Progress report on investigations of the Nudibranchiata of New England. GEORGE M. MOORE.

The author has undertaken a faunistic survey. Most information about New England nudibranchs comes from workers of the last century. Balch published a few records before 1910. It is apparent that the New England nudibranch fauna is not so varied, nor are the individuals so numerous, as on the coasts of the British Isles or on the Pacific coast of America. The following lists species found by the author chiefly from the intertidal zone, with dates and localities. Kodachrome photographs of living animals have been made of all except *Idulia* spp.

1. *Palio lessonii* (Orbigny). One from Rye, N. H., in tide pool, 7/22/50.

2. *Acanthodoris pilosa* (Abildguard) [= *Doris bifida* Verrill]. Very numerous on rocks and *Fucus* in intertidal zone at Dover Point, Great Bay, N. H., April and May, 1949 and 1950. A single specimen from the open coast at Kittery Point, Me.

---

[1] This work was supported by a grant from the Williams-Waterman Fund of the Research Corporation to Dr. George Wald.

3. *Onchidorus fusca* (Müller) [= *Doris bilamellata* L. of Alder and Hancock]. Not numerous but found in mature condition on rocks in tide pools throughout the year, in Great Bay, N. H. and on the open coast from York, Me., to Rye, N. H. Small immature specimens very numerous during late July and August, 1950, in same localities.

4. *Onchidorus aspera* (Alder and Hancock). Collected during fall, winter and spring on open coast from York, Me. to Rye, N. H.

5. *Dendronotus frondosus* (Ascanius) [= Dendronotus arborescens (Müller)]. On rocks and *Fucus* in intertidal zone in spring (few) and summer (numerous) in Great Bay, N. H., and on coast from York, Me. to Rye, N. H.

6. *Idulia coronata* (Gmelin) [= *Doto coronata* Gmelin]. At Newcastle, N. H. on *Sertularia* (7/26/50) and at Woods Hole, Mass. on *Tubularia* (8/16/50).

7. *Idulia pinnatifida* (Montagu). In Great Bay, N. H. on *Sertularia* (7/15/50).

8. *Coryphella rufibranchialis* (Johnston)
9. *Coryphella rufibranchialis chocolata* Balch } Most numerous of eolids collected. Abundant on rocks in tidepools on open coast from York, Me. to Rye, N. H. from late April through June. Few in Great Bay, N. H. Balch's variety with dark ceratia composes about 25% of population. His dark variety intergrades with the red.

10. *Eubranchus pallidus* (Alder and Hancock) [= *Galvina picta* (A&H)]. Collected twice (York, Me. and Rye, N. H.) April, 1950.

11. *Tergipes despectus* (Johnston). On *Sertularia*, Newcastle, N. H. (8/10/50).

12. *Cratena pilata* (Gould). On Pennaria at Woods Hole, Mass., Aug., 1950.

13. *Cratena aurantia* (Alder and Hancock). On *Tubularia* at Woods Hole, Mass. (6/21/50 and 7/3/50), Isles of Shoals, Me. (7/25/50), Great Bay, N. H. (8/19/50).

14. *Aeolidia papillosa* (L.). Three specimens at Woods Hole, Mass. (June, 1949) and York, Me. (April, 1950).

PAPERS READ BY TITLE

The effect of Janus Green B on cleavage and protoplasmic viscosity. Robert Day Allen.

Janus Green B (0.2 to 0.9 milligrams per cent) causes marked differential delay or even complete inhibition of cleavage in the eggs of the sea-urchin Arbacia, the sand-dollar Echinarachnius, the clam Mactra, and the worm Chaetopterus when applied three minutes (in Mactra, Arbacia and Echinarachnius) or five minutes (Chaetopterus) after fertilization. Earlier application of the dye usually caused total inhibition, whereas later application brought about little or no delay or inhibition. Cleavage was delayed in Arbacia eggs up to twenty-five minutes, in Echinarachnius fifteen, Mactra ten, and Chaetopterus less than ten. In Chaetopterus eggs there is a greater tendency toward inhibition rather than delay.

Fertilized eggs allowed to remain in low concentrations of the dye never passed the early gastrula stage, and usually stopped at the four or eight cell stage. Eggs fertilized in the dye seldom cleaved and were mostly abnormal. The inhibitory effects of Janus Green B were irreversible; inhibited eggs did not recover when returned to sea-water.

Janus Green is listed by Jorpes (1947) as an anticoagulant for blood. Consequently, protoplasmic viscosity determinations were made by the centrifuge method (Heilbrunn and Wilson, 1948) at 34, 38 and 40 minutes after fertilization at 21°C. Protoplasmic viscosity was still low at 34 and 38 minutes in the experimental eggs, although at this time the control eggs in sea-water showed typical mitotic gelation. At 40 minutes gelation had begun in some of the experimental eggs.

Growth of pedigreed strains of Paramecium caudatum and P. aurelia on a non-living medium. W. D. Burbanck.

Using Gilman's *Paramecium caudatum* (Number I) and Sonneborn's *P. aurelia* (Stock 51), 3 day mass cultures were run with daily counts. Single washed animals (Parpart's Method) were introduced into 1.0 ml. of three different media. Hard glass watch glasses were used and kept in Petri dish moist chambers. All runs were maintained at 25.5 ± 0.5°C. Tests for contamination were negative.

Living *Aerobacter aerogenes* (Sonneborn's Number 41 Aa) grown on lettuce infusion served as food for control paramecia and killed, as one ingredient of food for ciliates grown in a non-living medium. For control animals, 24 hour *Aerobacter* was diluted one-half with distilled water. The two non-living media consisted of killed *Aerobacter* with equal parts of 0.5% Anheuser-Busch yeast autolysate. In one case (van Wagtendonk's medium) bacteria were killed by autoclaving at 15 pounds for 20 minutes, while in the other case, by subjecting them to 60°C. for 10 minutes.

The following results were obtained:

	No. of runs	Av. daily div.-rate	Highest daily div.-rate
Paramecium caudatum			
Control	8	0.5	1.0
In three of the eight runs there were no divisions. *Aerobacter* overgrew paramecia.			
van Wag.'s medium	14	0.3	0.6
In no case did the animals divide the first day. In three days, 3 divided twice, 5 once, and 6 survived but did not divide.			
Bacteria killed at 60°C.	19	1.0	1.3
In all but two cases, animals divided the first day.			
Paramecium aurelia			
Control	8	2.7 plus	3.0 plus
van Wag.'s medium	16	0.9	1.3
Bacteria killed at 60°C.	16	2.2	2.5

Although the lettuce-bacteria-autolysate medium is a complex one, division-rates obtained when bacteria were killed at 60°C. were quite constant and repeatable. Particularly in the case of *P. aurelia* sufficient numbers of animals were obtained in three days to provide material for a variety of experiments.

Phosphorus compounds in the sea urchin and starfish eggs. EDWARD L. CHAMBERS. No abstract submitted.

Enzymic determination of adenosine triphosphate in the unfertilized and fertilized egg of the starfish. EDWARD L. CHAMBERS. No abstract submitted.

Observations on maturation of starfish eggs in which the nucleolus has been removed from the germinal vesicle. EDWARD L. CHAMBERS. No abstract submitted.

Viability of Arbacia gametes in relation to time. RALPH HOLT CHENEY.

In an attempt to reduce the slaughter, which is a factor in the current scarcity of *Arbacia punctulata* in the Woods Hole region, several investigators (Palmer, 1937, *Physiol. Zool.*, **10**: 352; E. B. Harvey, 1939, *The Collecting Net*, **14**: 180; 1940, *Biol. Bull.*, **79**: 363; Pequegnat, 1948, *Biol. Bull.*, **95**: 69; Tyler, 1949, *The Collecting Net*, **19**: 19) have suggested ways and means, primarily through the injection of isosmotic KCl (0.5 Molar), of "sexing" the individuals as to their maturity, for selective storage, and for the shedding of successive batches of eggs and sperm over a period of six weeks without sacrificing the life of the animal.

It may be helpful to Arbacia investigators, and in the interest of conservation, to report that during the past several summers, incidental to a study of the life span of Arbacia gametes *per se* under different concentrations of certain drugs, it has been observed that the eggs and sperm can be utilized satisfactorily as "fresh" for at least an eight hour post-shedding period in the case of the eggs and for a minimum of a 24 hour time lapse in the case of the sperm. It is unnecessary to collect gametes from (or to sacrifice) additional animals for preliminary experiments and for most types of data with the exception of the final critical studies in respiration.

The following procedure provides both "fresh" eggs, as determined by their 100% fertilizability; and, "fresh" sperm, as determined by their ability to fertilize; and, by the normalcy of the fertilized egg to develop in normal time and form into the pluteus larva. Eggs or sperm were shed into stender dishes containing 40 cc. of sea-water, allowed to remain in a concentrated, undisturbed mass, and with the cover allowing a small air space, were placed so that running sea-water surrounded the dishes up to one-half their depth. For use, two cc. of sea-water eggs (taken each time from the edge of the concentrated mass shed) were mixed with two drops of sea-water sperm (likewise obtained from the concentrated mass shed) or one drop of "dry" sperm, in a stender dish containing 40 cc. of sea-water. This procedure results in the formation of the fertilization membrane and the development into the larva in normal time and form in 100% of the eggs under these conditions. They respond in a manner precisely equivalent to the results seen by mixing freshly-shed gametes.

Sampling eggs of various ages (subsequent to shedding from one female) were mixed immediately and after 1, 2, 4, 8, 12, 16, 20, 24, 30, 36, and 48 hours, with sperm of the same post-shedding age (obtained from one male); and, also separate eggs of these same ages were mixed with fresh sperm. Likewise, sperm of these same ages were employed to fertilize fresh eggs. Tabular expression of the experimental statistics is given below.

Viability time table for Arbacia gametes

Post-shed. age of sperm in hrs.	Post-shedding age of egg in hours→							
			8	12	16	20	24	30
fresh	Immediate mixing of fresh egg and fresh sperm gives 100% fertiliz. membrane*	4-hr. egg ×fresh sperm results in 100% fertiliz. membrane	100% F.M.	100% F.M.	90–100% F.M.	90% F.M.	0–1% F.M.	No F.M.
4	Fresh egg ×4-hr. sperm 100% F.M.	4-hr. egg ×4-hr. sperm 100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	90% F.M.	0–1% F.M.	No F.M.
8	100% F.M.	100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	90% F.M.	0–1% F.M.	No F.M.
12	100% F.M.	100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	90% F.M.	0–1% F.M.	No F.M.
16	100% F.M.	100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	90% F.M.	0–1% F.M.	No F.M.
20	100% F.M.	100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	90% F.M.	0–1% F.M.	No F.M.
24	100% F.M.	100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	80%	0–1% F.M.	No F.M.
30	100% F.M.	100% F.M.	100% F.M.	100% F.M.	90–100% F.M.	75%	0–1% F.M.	No F.M.
36	75% F.M.	75% F.M.	75%	75%	75%	50%	0–1% F.M.	No F.M.
48	No F.M.	No F.M.	0%	0%	0%	0%	0%	0%

* F.M. formed typically and development occurs normally.

Results indicate: (1) The functionally normal, post-shedded life span of the unfertilized egg is appreciably less than that of the sperm. (2) For routine preliminary experiments, and with the exception of critical respiratory studies, eggs may safely be considered as well within the limits of dependability to function as physiologically "fresh" certainly for as long as eight hours and the sperm for twenty-four hours after shedding under the conditions of maintenance and use as stated above. One hundred per centum, or the equivalent of the control in any given batch of eggs, of the fertilizability of the eggs; and, of the fertilizing power (capacity) of the sperm, is retained during these periods and the subsequent development is normal in time and form.

The practice of the above procedure would enable zealous investigators, using large numbers of *Arbacia punctulata* gametes, to conserve their time and aid in the conservation of the species without jeopardizing the accuracy of their work. Similar conservation of other sea-urchin species may be worthy of consideration also by investigators at other Marine Laboratories.

The cytochrome oxidase and succinic dehydrogenase contents of squid (Loligo pealii) axoplasm and other nervous tissues. S. J. Cooperstein and Arnold Lazarow.

Previous studies on dog nerve indicate that whereas the concentration of succinic dehydrogenase is below the sensitivity of the method used, measurable amounts of cytochrome oxidase are present. Since these two enzymes are almost invariably present together in tissues, we have studied their distribution in the giant axon and in other nervous tissues of the squid.

Cytochrome oxidase was measured spectophotometrically by following the rate of oxidation of reduced cytochrome *c* at 550 mμ. Succinic dehydrogenase was determined by following the rate of reduction of oxidized cytochrome *c* in the presence of sodium succinate and cyanide (Cooperstein *et al.*, 1950). The right giant axon was dissected and cleaned and a sample of axoplasm weighing between 2.00 and 5.00 mgs. was extruded and analyzed. The left giant axon was similarly dissected and the enzyme content of the whole nerve determined. Samples of fin nerve, stellate ganglion, muscle, and a composite sample of the fibrous sheath plus small nerves surrounding the giant axon were also studied. The enzyme activity was expressed as Δ log [cytochrome *c*] min./mg. wet weight of tissue. Five samples of fin nerve and 6–8 samples of the other tissues were analyzed.

Significant quantities of succinic dehydrogenase were found in squid axoplasm (.108) compared to dog nerve which contains $<$ .012 (limit of sensitivity). Similar concentrations were found in the intact giant axon (.120), fin nerve (.113), and fibrous sheaths plus small nerves (.112). Muscle and ganglion contain significantly greater amounts of this enzyme (.155 and .227, respectively).

The cytochrome oxidase content of axoplasm (.395) was the same as that of intact giant axon (.375), fin nerve (.360), and fibrous sheaths plus small nerves (.410), but significantly less than muscle (1.53), and ganglion (2.12).

The cytochrome oxidase content of squid stellate ganglion is over five times that of dog spinal ganglion; the activity of the giant squid axon is 3.6 times that of dog nerve. The succinic dehydrogenase content of squid stellate ganglion is 1.74 times that of dog spinal ganglion.

Comparative studies on the cytochrome oxidase and succinic dehydrogenase content of toadfish (Opsanus tau) and rat kidneys. S. J. Cooperstein and Arnold Lazarow.

The cytochrome oxidase and succinic dehydrogenase contents of various tissues were determined spectrophotometrically by measuring the respective rates of oxidation and reduction of cytochrome *c* under standardized conditions. Samples of tissue were removed from 10 toadfish and 8–10 rats and the enzyme activities per mg. wet weight were compared for corresponding tissues.

In most tissues studied the ratio of the cytochrome oxidase concentration in the rat to that of the toadfish ranged from 0.97 to 1.66 (brain, 0.97; muscle, 1.06; liver, 1.38; and heart, 1.66). By contrast the cytochrome oxidase content of rat kidney was 3.13 times that of the toadfish.

Similarly, in the case of succinic dehydrogenase the ratio of enzyme concentration in rat tissue to that in toadfish ranged from 3.0 to 4.19 (brain, 3.00; heart, 3.03; muscle, 3.32; and liver, 4.19). On the other hand this ratio was 9.93 in kidney.

Thus the relative deficiency of these enzymes in toadfish kidney as compared with rat kidney is much greater than in other tissues. Whether this striking difference can be correlated with the aglomerular condition of toadfish kidney, with the marine habitat, or with the presence of gills which may take over a part of the renal excretory function, awaits further investigation.

Dividing Arbacia eggs as test objects for the effects of cortisone and desoxycorticosterone on cell division, potassium permeability, and glucose utilization. IVOR CORNMAN.[1]

Marine eggs would be a convenient tool for examination of the mechanism of adrenal steroid action, if the eggs could be made to exhibit any of the responses observed in mammalian systems. Division of Arbacia and Echinarachnius eggs is retarded by cortisone at 10–40 mg./L and desoxycorticosterone (DOC) at 5–10 mg./L. Both completely suppress Echinarachnius cleavage at 100 mg./L. DOC glucoside, although more soluble, is inactive at 1000 mg./L. Using concentrations at and below the slightly inhibitory level, the steroids were tested in situations with some resemblance to conditions under which they are active in the mammalian body. When Echinarachnius eggs are exposed to both steroids simultaneously, the delay is additive. The KCl-resistant and KCl-susceptible phases of the fertilized Arbacia egg (Chambers) are not altered by DOC, nor is the recovery in sea water following KCl-blocking of division. Thus DOC exhibits no tendency to permit K penetration during the resistant phase, does not prevent K penetration during the susceptible phase, and does not prevent K escape when the eggs are returned to sea water. Cortisone or DOC alone, glucose alone, or cortisone + glucose do not accelerate division or embryogenesis. An influence of glucose on the dividing egg can be manifested by means of colchicine, which becomes less inhibitory in the presence of glucose. This glucose effect is not enhanced by cortisone. The systems within the egg conceivably are remote from situations prevailing within the mammalian body. Therefore, the absence of demonstrable effects in echinoderm eggs sheds little light on the mechanism of action of the adrenal steroids.

Growth of Campanularia colonies. SEARS CROWELL AND MALCOLM RUSK.

Colonies of *Campanularia flexuosa* have been kept growing in the laboratory for several weeks by using the following procedure. A stem with from 10 to 25 hydranths, from a freshly collected colony, is tied by a thread to a glass slide. Such a stem develops attaching stolons in a day or two. From these stolons new stems (hydranths and hydrocauli) arise. The slide is placed in a rack in running, filtered sea water.. Newly hatched nauplii of Artemia are fed to the specimens twice daily.

The average time required for the development of a new hydranth, once its production was started, was 24 hours at 20–22°C., and 37 hours at 17°C. However, colonies doubled their mass in 3½ days at 17°C. as against 6 days at 20–22°C. Thus more hydranths were produced at a time at the cooler temperature.

Gonangia were produced at 17°C. but not at 20–22°C. When a gonangium was produced there was nearly always a (compensatory?) regression and resorption of the hydranth nearest the developing gonangium.

If a colony can show regulative or compensatory growth when its typical composition is altered, one would expect that the production of new stolons and stems would be more rapid in a colony from which nearly all of these components had been removed. Conversely, terminal growth from stems would be more rapid when their tips had been removed, leaving a colony with an excessive stolon system. Amputated colonies showed no tendency toward regulation in the manner suggested. Growth of new structures went forward from each locus of growth just as if no alteration of the composition of the colony had been effected. This result parallels those of Berrill who finds that the nature of the structure produced is dependent on slight differences in the morphology of the loci where their proliferation begins.

Phosphatase activity in Tetrahymena. ALFRED M. ELLIOTT AND ROBERT L. HUNTER.

Information concerning the phosphatase activity among the protozoa is even more scanty than for the invertebrates in general. Kind (*Biol. Bull.*, 1949, **97**: 262) found a "progressive increase in alkaline phosphatase activity with increasing biological complexity" among several marine invertebrates. It seemed important to examine these enzymes in at least one member of the first phylum of animals. *Tetrahymena geleii* was selected for investigation because its physiology and biochemistry is rather well known and its possibility as an experimental animal of importance shows promise.

---

[1] Aided by a grant from Bache Fund of the National Academy of Sciences and by an American Cancer Society institutional grant to the Warwick Memorial for Cancer and Allied Diseases, George Washington University.

When washed organisms (approximately 10,000/ml.) were suspended in distilled water containing 0.001 *M* adenylic and cytidylic acid (separately) at pH 6.0, 50% of the phosphorus was released into the supernatant in 45 hours of incubation at 24°C. Hence phosphatases are present in Tetrahymena.

Following the same procedure, but removing one-half the contents of the flask after 14 hours of incubation and filtering the protozoa, the phosphorus level remained constant in the filtrate, indicating that the enzymes were confined to the organism itself.

Similarly the pH optima were found to be approximately 5.2 and 8.7, being considerably more active in the acid range, which would be expected in view of Kind's observations on invertebrates.

By employing a synthetic medium (*Physiol. Zool.*, 1950, **23**: 85) which contained nucleic acid components as substrate and no inorganic phosphate added except that inadvertently included with protogen, it was found that phosphorus release proceeded rapidly prior to the onset of log growth, after which phosphate uptake obscured the picture. Total phosphorus readings of both supernatant and cells gave constant readings within an error of 5%. For several hours following log growth the organisms continued to take up phosphate at the same rate as during log growth.

"Spot" beats in deganglionated Limulus hearts. W. E. GARREY.

Removal of the median dorsal ganglionic cord stops the normal rhythmic beat of the heart of Limulus instantly and permanently. Twenty-four hearts were so prepared *in situ*. In 18 of these, subsequent examination revealed a few, small, discrete irregular areas of slow rhythmic contraction which were sharply localized; contractions which did not spread throughout the syncytial myocardium. Eleven of these contractile spots were located on either the first or second segment which are non-ganglionate. Heat applied to these spots did not accelerate their rhythm, the genesis of which, therefore, was not local or myal. A source of remote control was sought by exploring the dorsum of the posterior segments with a warm test tube. In 8 of the 11 instances, localized spots were found on the homolateral side of the posterior segment which accelerated the rhythm of the distant muscular contraction. The contractions stopped upon section of the fibers of the lateral nerve. A secondary extra-ganglionic neural mechanism is clearly involved in the "spot" contractions.

Microscopic examination of the localized neurogenis centers revealed small bipolar cells similar to those seen in the median ganglionic cord. No large, "pace-maker," nerve cells could be identified.

The effect of P^{32} on the division time of Arbacia eggs. J. W. GREEN AND J. S. ROTH.

Arbacia eggs were placed in sea water containing P^{32}. After an hour the eggs were fertilized and counts made, at intervals, of the first and second cleavages. The sea water was prepared by evaporating carrier free isotope solutions to dryness and then taking up the residue in sea water. Concentrations of 50, 5, and 0.5 μc of P^{32} per ml. were compared to an isotope free control. Preliminary results showed that the first and second cleavages were significantly delayed by the highest concentration of isotope. The intermediate and low isotope concentrations gave respectively smaller, but possibly significant, delays in both cleavages also. It is planned to extend the experiments by using different exposure times, concentrations and types of isotopes.

Staining procedures for whole mounts of the spinning glands of Habrobracon larvae. D. S. GROSCH.

In an attempt to throw light upon the organization and nucleic acid relationships of the multinucleate units which comprise the spinning glands of the parasitic wasp Habrobracon (Grosch, 1950, *Proc. N. C. Academy of Science*), many of the stains in common usage have been employed. It was found that only by the Feulgen method (Rafalko modification) are preparations produced which allow unobscured examination of nuclear elements. Stains such as haemalum and haematoxylin, commonly considered nuclear stains, are of little value because they give deep color to spinning gland cytoplasm. This disadvantage is found also for orcein and aceto-carmine; furthermore, technique which breaks the nucleus to release its contents is not recommended because of the large number of minute rod-shaped objects which resemble the chromosomes of a Hemipteran type somatic nucleus rather than the small number of large chromosomes of the Dipteran nucleus. An examination of slides containing series of glands from larvae of different ages stained with toluidine blue or Unna-Pappenheim pyronin-methyl green suggests that cytoplasmic basophilia increases during the four larval instars until the time of functional activity. This would indicate an increase in RNA during the development of the glands. Toluidine blue gives more repeatable results. The orange G component of Flemming's triple stain produces a very distinct histological picture of the arrangement of structural components, especially the trachea. Eosin was of little value.

Starvation studies with Habrobracon. II. Haploid males, diploid males, and diploid females compared in longevity. D. S. Grosch and A. M. Clark.

Recently, using the two more common animal types, Grosch (*Biol. Bull.*, 1950) demonstrated that gentically diploid females survive the gentically haploid males under conditions of complete starvation. This difference was more pronounced at lower temperatures and there was a distinct positive correlation between wasp size and longevity. The present report extends these findings to the genetically diploid males. The three sex types (haploid males, diploid males, and diploid females) were obtained from a cross involving stock 25. Offspring 2.2 to 2.7 mm. in length were collected and stored one animal per vial. Survival time in hours, as given by the mean with its standard error, at incubator temperature (30°C.) was 147.20 ± 2.69 for haploid males, 134.30 ± 5.57 for diploid males, and 154.28 ± 2.78 for females; at room temperature (20–25°C.) survival time in the same terms was 181.20 ± 4.08 for haploid males, 177.84 ± 5.28 for diploid males, and 223.20 ± 3.84 for females. These results in which the diploid male is the least viable type indicate that genetic diploidy *per se* is not related to the longevity but that a sex difference is involved. This is consistent with the hypothesis that during starvation females have a somewhat different basis of metabolism associated with oosorption.

Variable sensitivity of Chaetopterus eggs to ultraviolet light. Paul Randolph Gross.[1]

Sea-urchin eggs are more sensitive to ultraviolet light during the first 30–40 minutes after insemination than they are later in the mitotic cycle (Giese, 1947; Blum, 1950; etc.)

Fertilized eggs of the worm Chaetopterus were irradiated at various points in the mitotic cycle in order to determine whether these eggs also show variable sensitivity.

In these experiments, the source of radiation was the Westinghouse Sterilamp. The spectrum of this apparatus has a maximum at 2537 Å. A constant dose was employed in all experiments (3 minutes: 8640 ergs/mm.^{2}). With this equipment, little or no cytolysis was encountered, and the irradiated eggs ultimately cleaved in the same numbers as did the controls.

Assuming a cleavage time of 57 minutes for a typical control, the sensitivity varies thus:

The most sensitive part of the cycle lies in the time from fertilization to 30 minutes afterward. Our standard dose at any point in this period consistently induced cleavage delays of from four to eleven minutes.

The period from 32 to 40 minutes is relatively insensitive. Here the standard dose usually failed to produce any delay at all, and occasionally effected a delay of one or two minutes.

During the time from 40 to 50 minutes, an increase in sensitivity seems to take place. Irradiation in this period regularly produced cleavage delays of from three to six minutes.

We have not yet been able to obtain reliable values for the period from 50 minutes after fertilization to the time of cleavage.

These facts might suggest a correlation between the sensitivity to ultraviolet light and the viscosity changes demonstrated by Heilbrunn and Wilson (*Biol. Bull.*, 1948, **95**: 57), or they may be related to nuclear events which take place at the same time.

---

[1] Aided by a grant from the U. S. Public Health Service.

Photography of Arbacia punctulata eggs with infrared light. ETHEL BROWNE HARVEY AND GEORGE I. LAVIN.

Photographs of the stratified centrifuged egg of *Arbacia punctulata* taken with infrared light (8000–10,000A°) are similar to those taken with visible light, including the mitochondrial layer. The yolk granules, however, appear coarser and the red pigment granules appear similar to the yolk granules, not darker as with visible light. In photographs of the plutei also, taken with infrared, the red pigment spots, so characteristic with visible light do not show at all; otherwise the photographs with infrared and visible light are similar.

Photographs of the unfertilized Arbacia egg and of all stages of the fertilized whole egg and red "half-egg" have been taken with infrared light. The nuclei of resting stages, the streak of the very early prophase, the dumb-bell shaped mitotic figure of the metaphase, and the separated spheres of the telophase stand out with remarkable clearness as white areas in the dark granular-appearing egg. Cleavage planes are prominent as white lines. The nuclei of the many-celled early and late blastulae are so clear that they might readily be counted. A better configuration of the nuclear phenomena in the fertilized red "half-egg" is obtained than with visible light. The picture is not obscured by the red pigment granules which apparently do not absorb this wavelength of light. Owing to the penetrating power of infrared light, it is excellent for the general configuration of the mitotic figures, but owing to its poor resolving power, is not good for detailed structure.

Luminescent reactions in the "railroad worm," Phrixothrix. E. NEWTON HARVEY.

The luminous beetle, Phrixothrix, with both a red and yellow light organ is so rare that knowledge of its chemiluminescent processes must be gathered little by little. At the General Scientific Meeting in 1949, I reported that sodium adenosine triphosphate (ATP) does not revive the luminescence of extracts of either the red or the green light organs, a result contrary to the behavior of the fire-fly, *Photinis pyralis.* The experiment has been repeated and confirmed in 1950 on another specimen of Phrixothrix sent by Dr. P. Sawaya and the reason for the negative result discovered. Luciferin disappears quickly in the extract of Phrixothrix, for addition of purified fire-fly luciferin (kindly supplied by Dr. W. D. McElroy) to the extract of the yellow light organs containing ATP does excite luminescence. Adding fire-fly luciferin to the extract of the red light organ plus ATP gave only a very faint luminescence, if any.

A recent study of Jamacian fire-flies by McElroy and Harvey (to be published shortly) indicates three types. In some species (1), ATP disappears quickly in an extract of lanterns and light will reappear on adding ATP. In others (2) luciferin disappears quickly and no light can be obtained on adding ATP but luminescence will occur on adding luciferin. In still other species (3), luciferase disappears rapidly and neither ATP nor luciferin will restore the light but addition of luciferase from another fire-fly will do so. The yellow light organs of Phrixothrix appear to belong in the second category. Further work will be necessary to characterize the red luminescence. The red color of the light suggests oxidation of a porphyrin derivative rather than a compound similar to fire-fly luciferin. (See Harvey, *J. Cell. Comp. Physiol.*, 1944, **23**: 31; 1945, **26**: 185.)

Nitrogen deficiency and coloration in red algae. FRANCIS HAXO AND PHYLLIS STROUT.

Many of the Rhodophyta collected in the late summer from inshore waters are decidedly bleached in appearance. They may be variously almost colorless, pale red, tan, yellow, or green. When brought into the laboratory and maintained in tap sea water enriched with KNO_3, such thalli undergo a definite and in some cases striking color change, usually to deep red or brown, resulting from an increased synthesis of plastid pigments. All red algae tested showed a color change in varying degree. These were: *Chondrus crispus, Agardhiella tenera, Ceramium rubrum, Polysiphonia, Champia, Lomentaria, Ahnfeltia, Chondria* (3 species), *Porphyra variegata, Hypnea,* and *Gracilaria.* Control thalli kept in unenriched sea water retained their bleached appearance.

The minimal concentration of supplementary KNO_3 required to effect a reddening in *Agardhiella* and *Ceramium* lies between 0.01 mM and 0.05 mM. At higher concentrations up to 5 mM a rather uniform reddening resulted, whereas 10 mM gave toxicity symptoms. The color changes were first noticeable within 24 hrs. and appeared complete within about 4 days.

The effect is specifically due to added nitrogen. KCl, phosphate, and ferric citrate were ineffective over a wide concentration range. NH_4Cl, $NaNO_2$, and NH_4NO_3 can each replace KNO_3 as sources of inorganic nitrogen for this reaction.

The photosynthetic rate of *Agardhiella* which had turned red under the influence of added nitrate was double that of a control portion of the same thallus kept in straight sea water. Development of fruiting structures (carposporangia) in *Agardhiella* and *Porphyra* was stimulated by supplementary nitrate.

The enzymes of the isolated islet tissue of the toadfish (Opsanus tau): cytochrome oxidase and succinic dehydrogenase. ARNOLD LAZAROW AND S. J. COOPERSTEIN.

It has been suggested (Lazarow, 1948) that the metabolic specialization of the beta cells for insulin synthesis predisposes these cells to the toxic action of alloxan. The reduction of alloxan and dehydro-ascorbic acid to dialuric acid and ascorbic acid respectively abolishes their diabetogenic effects. Since these compounds are reduced by certain enzyme systems while their reduced forms are reoxidized by oxidized-cytochrome *c,* we have studied the enzyme systems in the isolated islet tissue of fish.

The concentration of oxidized-cytochrome *c* in the cell is determined by the total cytochrome *c* present and by the enzymes which oxidize (cytochrome oxidase) and reduce (succinic dehydrogenase, etc.) cytochrome *c*. Cytochrome oxidase was measured spectrophotometrically by following the rate of oxidation of reduced cytochrome *c* at 550 mμ. Succinic dehydrogenase was determined by measuring the rate of reduction of oxidized cytochrome *c* in the presence of sodium succinate and cyanide (Cooperstein *et al.,* 1950). Samples of islet tissue weighing between 1.00 and 2.00 milligrams sufficed for the enzyme assay. The enzyme activity was expressed as Δ log [cytochrome *c*] /minute/mgm. wet weight of tissue and the average value for 10 animals was calculated.

The succinic dehydrogenase content of islet tissue (.085) was lower than that of brain (optic lobes) (.206), kidney (.239), liver (.412) and heart (1.373) but not significantly different from testis, gill, skeletal muscle, and ovary (.072–.085). The cytochrome oxidase content of islet tissue (.61) was significantly higher than that of muscle (.375) and gill (.465), but lower than that of ovary (.755), brain (.770), testis (.880), liver (1.98), kidney (2.02), and heart (3.50).

The ratio of succinic dehydrogenase to cytochrome oxidase in islet tissue (.139) was lower than gill (.170), muscle (.203), liver (.207), brain (.288) and heart (.392) and slightly higher than kidney (.119), testis (0.96), and ovary (0.96).

If the distribution of other cytochrome *c*-reducing enzymes parallels that of succinic dehydrogenase, these results would suggest the possibility that islet may have a high capacity for converting potential diabetogenic compounds into their active forms.

Are nutritive substances essential for hydranth formation in Tubularia? JAMES A. MILLER, JR.

The experiments of Barth (1938) and Rose and Rose (1941) showed that substances circulating in the coelenteron influence Tubularian reconstitution. Whether these substances are nutritive, inhibitory or both has not been fully answered.

The following experiments were designed to test whether there are nutritive substances circulating in the coelenteron which are essential for hydranth formation (Barth, 1938, 1940, 1944). The tips of eyedroppers, drawn to a diameter of about ½ mm., were inserted into the proximal ends of Tubularian stem segments 10 mm. long. Twenty stems impaled as described were inserted into a battery jar 27 cm. in diameter through which there was a constant flow of filtered sea water.

The water in the eyedroppers was maintained about 5 cm. lower than that in the jar by inserting a small tube, connected with an aspirator, into the large part of the eyedropper. In this fashion a constant gentle flow of sea water was maintained through the ten experimental stems. Controls were treated similarly but were not connected with the aspirator. Patency of the experimental stems was determined at intervals by observing the rise of water in the eyedroppers when the suction was stopped for half an hour.

Experiment 1. One of the experimental stems became plugged and was discarded. So also were two in which the coenosarc was carried up into the eyedroppers. All of the seven remaining stems reconstituted (100%). 70% of the control stems reconstituted.

Experiment 2. Two experimental stems became plugged and two lost their coenosarcs. The six remaining all developed hydranths (100%) as compared with 80% of the controls.

Since the flow of water removed any nutritive substances which might have been released into the coelenteron and since the experimental stems reconstituted as well as did the controls, it is concluded that there are no circulating nutritive materials which are essential for hydranth formation.

Activation of Nereis eggs by a detergent. W. J. V. OSTERHOUT.

Unfertilized eggs of *Nereis limbata* were exposed to 0.004 per cent duponol (chiefly sodium dodecyl sulfate) in sea water for 30 to 60 seconds. Jelly was extruded and in some cases segmentation occurred, and a small percentage of eggs formed trochophores which swam near the surface.

The procedure was as follows: Pasteurized sea water was used in all cases. The female was washed and was caused to shed in 250 ml. of sea water. The eggs were placed for 30 to 60 seconds in the solution of duponol in sea water and were then transferred to 250 ml. sea water where segmentation occurred in some cases. The temperature was approximately 25°C. Controls without duponol showed no segmentation.

No attempt was made to secure a larger percentage of trochophores since this was not the primary purpose of the experiments.

Relative solubility of the components of the Nereis egg. W. J. V. OSTERHOUT.

The egg is surrounded by a tough vitelline membrane which is resistant to dissecting needles. It does not dissolve when exposed for several hours to 5 M H_2SO_4 but it dissolves in less than 5 minutes in 1% duponol (chiefly sodium dodecyl sulfate).

After the disappearance of the vitelline membrane the cytoplasm spreads out in the solutions and the nucleus is exposed to the action of the duponol. It does not dissolve but remains for several hours with little change in appearance except for slight swelling.

The oil drops show no change. The other constituents of the cytoplasm become dispersed in the surrounding solution and their fate cannot be precisely determined.

Javelle water is prepared from chlorinated lime in the usual way using sea water in place of distilled water. It is allowed to stand until a precipitate settles out and then diluted with 3 parts of sea water. This acts somewhat like 1% duponol except that the nucleus shrinks and becomes opaque.

With undiluted Javelle water everything dissolves except the oil drops.

A cytological analysis of the effects of cyanide and 4,6-dinitro-ortho-cresol on the mitotic phases in Arbacia punctulata. ALLAN SCOTT.[1]

It has long since been demonstrated by Clowes and Krahl (*J. Gen. Physiol.*, 1936, **20**: 145), by Robbie (*J. Cell. Comp. Physiol.*, 1946, **28**: 305) and by others that the oxygen consumption of fertilized Arbacia eggs is inhibited by suitable concentrations of cyanide and is stimulated by the nitrophenols. Moreover, both reagents suppress cell cleavage. We have treated the Arbacia eggs in sea water solutions of these inhibitors in concentrations ranging from 5×10^{-2} molar down to 10^{-6} molar (pH 8.1). In every case we have prepared slides to check the mitotic condition.

The first few minutes of development in all strengths of both reagents is at a nearly normal rate and this is true even when eggs are pretreated to insure penetration. Eggs pretreated for fifteen minutes with 5×10^{-2} molar NaCN or even for one hour in 10^{-3} NaCN, will fertilize in the solution, raise a fertilization membrane, show sperm penetration, male pronuclear migration and pronuclear fusion by plus fifteen minutes, but the male pronucleus fails to swell. If immersed at progressively later stages, development continues correspondingly longer but we find no phase specific inhibition. Eggs treated with high concentrations in the early prophase never emerge from the prophase simply because it is a phase of long duration. In 5×10^{-2} molar NaCN and in 10^{-2} molar 4,6-dinitro-ortho-cresol, there is little development beyond the first fifteen minutes, which is at the near normal rate. In lesser strengths development continues very slowly. Thus eggs immersed in 10^{-4} NaCN at thirty minutes gave no cleavage by 142 minutes, but 53% by 5½ hours. Robbie found a maximum inhibition of oxygen uptake at about 10^{-4} molar NaCN.

---

[1] This study was made during tenure of a fellowship from The National Institute of Health, U. S. Public Health Service.

Eggs in 10^{-4} 4,6-dinitro-ortho-cresol at thirty minutes (crescent stage) arrive at prometaphase at fifty minutes, scarcely delayed over metaphase controls. At seventy minutes there are a few anaphases, at 107 minutes a few are cleaved and at 280 minutes: 20% are one cell, 58% are two cell, and 22% are three or four cell. Clowes and Krahl found that the optimum stimulation of oxygen consumption was close to 10^{-6} molar for this reagent. In 100 × this concentration we find slow development during 4½ hours.

Factors involved in the breakdown of the germinal vesicle in the egg of Chaetopterus pergamentaceus. Allan Scott and George LeBaron, Jr.

The development of the Chaetopterus egg is arrested at two stages; the first arrest is at the germinal vesicle stage and the second, at the first polar metaphase which persists normally until fertilization. We are here concerned with the factors responsible for the first arrest.

The passage to the first metaphase is perfectly normal in many different solutions. It is normal, for example, in 2 × sea water, in 50% sea water and in calcium free sea water. It is unaffected by isotonic KCl, $CaCl_2$ or NH_4Cl. The pH range is very wide, for eggs will go to the metaphase in sea water at pH 5.0 and at pH 9.0. Transfer to viscous solutions of ½% agar in sea water or 6% egg albumin (dialized against sea water) does not affect the movement to the metaphase, indeed the process is uninhibited in mineral oil. It is markedly slowed, but not inhibited by sea water at 7°C.; however, one minute at 42°C. inhibits germinal vesicle breakdown completely. Ninety-two per cent of the eggs of some females, ovulated into parapodial fluid, may remain in the germinal vesicle stage for at least 60 minutes. This figure may be as low as thirty-two per cent in other females.

The breakdown of the germinal vesicle is completely inhibited by strong NaCN (.05 *M*), and the reaction is reversible since eggs treated for as long as an hour will give good cleavage. Similarly there is a persistent germinal vesicle in .05 *M* sodium azide and in sea water saturated with H_2S. There is no inhibition by carbon monoxide in the light or in the dark, nor is the process inhibited by .05 *M* iodoacetic acid.

We feel that this development to the first polar metaphase resembles parthenogenetic activation in that a delicately poised reaction is triggered by many types of treatment including pressure. Apparently cytochrome oxidase is not involved in the mechanism and it remains to be seen what enzymes are being inhibited by the cyanide, azide and H_2S.

The influence of the glycolytic inhibitor iodoacetic acid on aging and on the potassium and sodium content of the egg cells of Mactra solidissima. George T. Scott and Hugh R. Hayward.[1]

The egg cells of Mactra undergo marked and peculiar morphological changes on aging over a twenty-four hour period. At about twelve hours they undergo a deep tubular indentation and assume a cup-like shape. At twenty to twenty-four hours a membrane is raised from the egg, and later the germinal vesicle disintegrates. Up to this time, according to Schechter (*J. E. Zool.*, 1941, **86**: 461), the eggs are fertilizable.

Associated with the aging process is a loss of potassuim and an uptake of sodium by the cells. The potassium begins to leave the cells at the time the indentation phenomenon becomes pronounced (9–12 hours), and concomitantly the sodium enters. At 24 hours, 70% of the potassium has escaped and the sodium content has more than doubled. These changes are described in the following table:

---

[1] Aided by a grant from the Biochemical Section of the Office of Naval Research with Oberlin College.

Age in hours	K meq. %	Na meq. %	% Indentation	% Raised membranes
2	12.7	14.6	0	0
4	12.5	19.4	0	0
6	13.1	16.0	4–5	0
9	13.1	15.9	10–12	0
12	9.1	22.7	40	0
15	6.0	25.2	15	85
23	3.6	38.3	0	90–95

The action of 0.001 M iodoacetic acid is such as to retard the development of the morphological aging signs and to cause the cell to maintain the original electrolyte levels for a longer time. The potassium then leaves the cell at a slower rate and the rate of entrance of sodium is also reduced. At about 36 hours the potassium and sodium content of the cells in iodoacetic acid resembles that of the controls. The action of the inhibitor on these cells is the direct opposite of the heretofore reported studies of its influence on potassium retention. This inhibitor causes a loss of potassium from erythrocytes, Arbacia eggs, brain cortex and yeast, presumably because of the inhibition of underlying glycolytic metabolism. It is possible that the slowing down of some metabolic mechanism by the inhibitor in the Mactra egg may retard the "aging" process.

Aqueous chlorophyll preparations from blue-green algae. Julius Silberger and Francis Haxo.

Tolypothrix lanata, *Scytonema crispum*, and *Oscillatoria* yield aqueous whole pigment extracts which are quite clear and give sharp absorption spectra similar to that reported by Emerson and Lewis (*J.G.P.*, 1942, **25**: 579) for *Chroococcus*. Addition of 15% $(NH_4)_2SO_4$ to the fluorescent extracts causes separation of a green precipitate after several hours. Most of the precipitate can be readily resuspended in water or pH 7 phosphate buffer to give green, nonfluorescent but slightly opalescent "solutions." Such initial preparations contain bound chlorophyll and carotenoids essentially free from phycocyanin.

Preliminary attempts have been made to purify and characterize the aqueous chlorophyll fraction from *Tolypothrix*. Crude extracts prepared by grinding or blending were cleared by filtration and low speed centrifugation. Fractional centrifugation between 2000 and 140,000 × gravity yielded a series of progressively smaller sediments, the major portion being thrown down below 20,000 g. The supernatant contained c-phycocyanin in solution. Absorption spectra of fractions obtained between 2000 and 80,000 g indicated uniform pigment composition. The curves are sharp and mainly characteristic of chlorophyll-a with the peaks displaced about 13 mμ toward longer wavelengths from those in methanol.

Addition of acetone or methanol to the resuspended green sediment caused flocculation of a colorless material which gave positive biuret and ninhydrin reactions for proteins. The extracted fat-soluble pigment consisted of chlorophyll-a and predominantly one epiphasic carotenoid. The suspensions are not coagulated by heat but show markedly altered absorption in the visible spectrum, the peaks shifting to shorter wavelengths.

It seems probable that such aqueous extracts contain chlorophyll-a and carotenoids bound together on the same particles, perhaps protein in nature, and not in true solution as is phycocyanin. Further studies of these preparations including their photochemical activity are planned.

A study of the development of the fin and trunk musculature in Elasmobranch fishes. William L. Straus, Jr.

Following the investigations of Balfour and others in the last quarter of the past century, it was generally accepted that in all vertebrates the striated musculature of both trunk and limbs is derived ontogenetically from the somites alone. Recent studies, experimental and otherwise, have demonstrated that the musculature of the extremities (possibly excepting some limb-girdle muscles) is of nonsomitic origin in tetrapod vertebrates (amphibians, birds, mammals; reptiles?), developing *in situ* from the limb-bud mesoderm. The view persists, however, that the entire trunk musculature is formed by the somites alone. Recent experimental studies on the chick (*Anat. Rec.*, 1948, **100**: 755; complete report in preparation) demonstrate that whereas the intrinsic back muscles are of somitic origin, the abdominal (ventrolateral) trunk muscles arise directly from the non-segmented mesoderm of the lateral plate. Experiments on larvae of the urodele amphibian, *Amblystoma* (Straus, unpublished), suggest similar origins of these muscles.

The classical studies of the development of the limb and trunk musculature of elasmobranch fishes were made by Balfour and others. The results of these studies, carried out entirely on normal fixed and stained specimens, has obviously influenced subsequent thinking. From the published work on elasmobranchs, it appears probable, although not certain, that the muscles of both the paired fins and the trunk are of somitic origin. If this is true, the development of the striated musculature differs among the several classes of vertebrates.

With this possibility in mind, the writer has been collecting normal developmental series of both the skate (*Raia erinacea*) and the smooth dogfish (*Mustelus canis*), with the view of re-studying the normal development of the musculature of the paired fins and trunk in elasmobranchs and comparing it with muscular development in other vertebrates. Such a re-investigation is clearly indicated.

An even more conclusive approach to the problem would be an experimental one, such as has been made in the chick and amphibians. The writer has, therefore, been studying living embryos of the skate toward this end. The reproductive habits of the skate would seem to make this animal a particularly favorable one for such studies (as by carbon marking and extirpation of somites, etc.). The inability at present to secure prolonged survival of the embryos (necessary because of the very long period of development in all elasmobranchs) has proved to be an obstacle to the application of experimental methods; but it is believed that this obstacle is not unsurmountable.

Studies of the carbohydrate metabolism of invertebrate tissues in vitro. Claude Villee, Robert Lichtenstein, Neal Nathanson and Brita Rolander.

A study was made of carbohydrate metabolism in tissues from certain marine animals cultured *in vitro,* to determine whether the utilization of glucose and the synthesis of glycogen could be demonstrated and whether insulin had any effect on these processes. The tissues, thin sheets of gill or muscle or slices cut with a Stadie microtome, were incubated in Warburg vessels in artificial sea water (Van't Hoff B with calcium) containing 200 mg. per cent glucose at 25 ± 1°C. As shown in the Table, glucose uptake and glycogen synthesis occurred in all tissues tested but the addition of insulin (0.5 unit/ml.) had no effect in increasing these processes in any save skeletal (branchial) muscle from the dogfish, in which a threefold increase was obtained. No differences were observed in dogfish muscle incubated in isosmolar mixtures of artificial sea water, glucose, and urea in concentrations of 0, 2.3, or 4.7 gm./100 ml. These results confirm the work of Florkin and Duchateau (1939) who were unable to demonstrate any *in vivo* effect of insulin on the blood sugar content of crayfish.

Tissue	Number of determinations	Initial glycogen*	Glycogen synthesized†	Glucose uptake†	Oxygen uptake†
Mactra muscle slices	5	.82	20.0	—	3.5
Mactra gill	15	.40	3.3	4.2	11.3
Pecten gill	10	.14	2.3	6.4	9.9
Pecten mantle muscle	5	.25	1.1	1.2	5.8
Mya gill	10	.29	2.3	2.8	12.5
Busycon white muscle slices	10	3.55	12.2	2.3	2.3
Busycon red muscle	2	1.76	—	—	—
Chaetopterus muscle	10	1.03	1.8	11.8	5.2
Thyone muscle	30	.61	2.6	4.7	1.4
Dogfish branchial muscle	14	.30	1.8	3.0	2.3
Dogfish smooth muscle	6	.18	1.8	—	4.0

* The figures given are mg. glycogen per 100 mg. (wet weight) tissue.

† The figures given are micromoles per hour per gm. (wet weight) tissue.

Phosphorus metabolism in embryonic and adult invertebrate tissues. CLAUDE VILLEE, ROBERT LICHTENSTEIN, NEAL NATHANSON AND BRITA ROLANDER.

Studies of phosphorus metabolism in embryonic and adult invertebrate tissues were made by culturing fertilized eggs and excised adult tissues in sea water plus radioactive phosphorus (P^{32}), added as inorganic phosphate. Fertilized eggs of the sand dollar, Echinarachnius and the sea urchin, Arbacia, were used. The adult tissues used were gills from the clams Mya and Mactra and the scallop Pecten. After incubation the tissues were fractionated by a modified Schmidt and Thannhauser method and the specific activities of acid soluble P, phospholipid P, total acid insoluble P, desoxypentose nucleic acid P, pentose nucleic acid P, and phosphoprotein P were determined. The order of activity in embryonic tissue was DNA > PNA > phosphoprotein, as in previous studies (Villee *et al.*, 1949). The order in adult tissues was phosphoprotein P > PNA > DNA, phosphoprotein phosphorus being turned over 10 to 40 times as fast as the phosphorus in DNA. The amounts of phosphorus in the several fractions in the adult tissues remained essentially constant over incubation periods up to twenty hours. Adult tissue has a large fraction (about 40%) of the total acid insoluble phosphorus present as DNA P, and this P is turned over only very slowly. In contrast, developing embryos have only a small fraction of the total acid insoluble P (1.5% in unfertilized eggs, 11% after 10 hours of development in Echinarachnius) present as DNA P, but this P is turned over quite rapidly.

The occurrence of a new variety containing two opposite mating types of Paramecium calkinsi as found in sea water of high salinity content. RALPH WICHTERMAN.[1]

Enormous numbers of *Paramecium calkinsi* repeatedly were found living and reproducing in pools of sea water of high salinity content ("evaporation pools") near the sea in the region of the sand dunes of West Barnstable, Massachusetts. Described previously from fresh and brackish water, this represents the first account of this species from this type of environment. The salt water in which the paramecia flourished showed the salinity to be 37.66 (as determined by the Woods Hole Oceanographic Institution); sea water around Woods Hole has a salinity of 31–32. Even in water of such great salt concentration, the paramecia possessed contractile vacuoles. Single specimens and conjugating pairs in the original fluid swim characteristically and conspicuously in right spirals, the only species of the genus to show this feature.

While the paramecia were cultivated successfully in an infusion made of desiccated lettuce and filtered sea water only, maximal growth was obtained by using two parts of standard lettuce infusion in distilled water and one part filtered sea water.

Since many conjugating pairs were found in the original collections, single specimens were isolated and many clonal cultures established to test for mating. All possible mixtures were made not only among the newly established clones but among others maintained in permanent culture for several years including the two Yale clones of opposite mating type. Up to the present time, only this one variety with its two opposite mating types, has been described for this species. The result of all possible mixtures of the newly established clones and Yale clones resulted in the discovery of a second variety containing two opposite mating types. As of now, there exists two non-interbreeding varieties each containing two opposite mating types.

A simple method for obtaining large numbers of fission-stages of Paramecium and certain other negatively geotropic ciliates. RALPH WICHTERMAN.[2]

By means of a new device, it is possible to obtain quickly and easily large numbers of concentrated dividing stages of Paramecium and certain other negatively geotropic ciliates. The method is based on the fact that dividing specimens not only move more slowly than typical vegetative specimens but once division has progressed to the point where a definite transverse constriction furrow is formed, the fission stages, especially late ones with their characteristically uncoordinated manner of swimming, tend to settle toward the bottom of the culture container.

---

[1] Aided by a grant from the Committee on Research and Publication, Temple University.

[2] For aid in the construction of the device, acknowledgment is gratefully made to Mr. James G. Graham, Jr., Mr. Michael Troisi and the Committee on Research and Publication, Temple University.

The device consists of a 4000 ml. Pyrex beaker from which the bottom has been cut off and ground smooth. To this opening is fused a Pyrex funnel with a 60° angle and of the same diameter as the opening at the bottom of the cut beaker. To the stem of the funnel, there is joined the inner member of a 19/38 standard taper, ground-glass joint while the outer member is fastened to a glass stopcock having a 5 mm. opening. The ground-glass joint is held firmly in place with two coiled springs. The glass device is set on a stand consisting of a wooden base and upright steel arm which holds two adjustable, rubber-covered brass rings. The smaller ring supports the funnel section and the larger ring supports the beaker section.

The device is used in the following manner: a rich culture (approximately 4 liters or less) of species pure *Paramecium caudatum* is placed in the unit. At the peak of the logarithmic growth phase of the culture after the paramecia are supplied with ample bacterial food, three or more generations per day are produced. By simply opening the stopcock from time to time and drawing off a few cc. of culture fluid, great numbers of fission-stages may be obtained in fairly concentrated condition.

It should prove of value to those who require concentrated numbers of division stages in studies dealing with cytology, irradiation and action of chemicals.

Proteolytic enzymes in the eggs of the clam, Mactra solidissima. A. A. Woodward.

Intracellular proteinases are being investigated in the effort to elucidate the mechanism of protoplasmic clotting. In this study the eggs of the large clam, *Mactra solidissima,* were used. Eggs taken from the dissected ovaries were repeatedly washed to eliminate the possibility of contamination by alimentary proteases. The tough outer membranes were softened by treatment with isotonic urea, and the eggs were then washed and homogenized in a medium containing 0.34 M potassium chloride, 0.105 M potassium citrate, 0.053 M sodium chloride, buffered to pH 8.0 with 0.005 M imidazole. The hemoglobin method of Anson (1938) was used to assay homogenates or their fractions for proteinase activity.

A graph of pH against enzyme activity reveals prominent proteinase activities with pH optima at pH 7.5–7.6 (Enz I) and at pH 3.5–4.0 (Enz II). Preliminary experiments indicate that Enz I is activated by Ca ions. Enz II is apparently not affected by Ca ions.

The method of centrifugal fractionation was used to achieve a degree of separation of the various protoplasmic particulates. Four reasonably distinct fractions may be obtained: (1) Large granules, easily resolvable under the microscope (234 × g for 10 min.); (2) intermediate granules (234 to 446 × g for 30 min.); (3) small granules (25,000 × g for 90 min.), unresolvable or invisible under the microscope; and (4) granule-free supernate.

Fractions (1) and (2), tentatively identified as yolk platelets and mitochondria, respectively, have negligible proteolytic activity. Enz I activity appears in fraction (3), small granules, and in fraction (4), clear supernate; fraction (3) has greater specific enzymatic activity but less total amount of activity than fraction 4. Enz II activity is almost entirely associated with fraction (4), the clear supernate.

Water and biological morphology. Dorothy Wrinch.[1]

The fundamental importance of water throughout biological studies needs little emphasis and it is to be anticipated that water molecules play a decisive role in all living systems. Gradually accumulating experimental data from various fields offer suggestions as to what this role may be.

The morphological potentialities of the HOH molecule are demonstrated by the structure analyses of ice. Each HOH molecule in such crystals surrounds itself with 4 of its fellows which it holds by means of OH..O hydrogen bridges at definite distances in definitely related directions—at the vertices of a regular tetrahedron, at distance 2.7A. The long known tridymite-like ice has hexagonal symmetry. In the recently discovered cristobalite-like ice (*Z. f. Krist.,* 1944, **105**: 279) the HOH molecules again array themselves tetrahedrally, but now with cubic (T_d) symmetry, a fact of great moment in connection with protein structure, since a close relationship between skeletons of protein molecules and cubic (T_d) networks has been surmised (*Biol. Bull.,* 1945, **89**: 192). It is also known from the case of (*e.g.*) oxalic acid dihydrate that individual water molecules can clamp other molecules together in hydrogen bridge circuits. Particularly interesting are the 5– and 29–hydrates of phosphotungstic acid in which not only individual waters but "crystal" fragments such as crumpled hexagons and even massive water clusters of considerable size and precise (actually cubic T_d) architecture function in the same way. The precise interpretation of the numbers of water molecules per anion which result ($5 = \frac{1}{2}(6+4)$, $29 = 1+4+6+6+12$) is a beautiful example of the impact of number theory on a structure problem in precisely the manner in which it may be expected also to uncover the significance of particular proportions of individual amino acids which have been found in certain proteins.

---

[1] This work is supported by the Office of Naval Research under contract N8onr-579.

The body of results, of which examples have been given, make it highly probable that water molecules play a definitely structural role in living organisms. When the water complements are relatively low, they will undoubtedly act as clamps which hold other molecules in definite orientations, at definite distances apart. When, however, there are vast complements of water, we may anticipate also the formation of clusters of the most precise and varied morphologies. Just as in ice where these small molecules are capable of maintaining a precise alignment over macroscopic dimensions, so too in cytoplasm they may play a large part in holding other molecules and ions, and even massive protein structures, in structurally significant arrangements, extending over a complete living cell.

Water and biological function. DOROTHY WRINCH.[1]

A succinct demonstration of the functional role of water in living systems has recently been recorded (*Nature,* 1950, **165**: 938). A culture of encysted young *Amoeba proteus* was allowed to dry up completely until "nothing was left except a thin layer of greenish dust." Water was added and five months later young amoebae were observed and "it became clear that the culture was re-establishing itself." In the results obtained in x-ray crystal studies of proteins (*Cold Spring Harb. Sym. Quant. Biol.,* 1949, **14**: 65) and physico-chemical investigations (Svedberg and Pedersen, *The Ultracentrifuge,* 1940) we may find suggestions as to the interpretation of these facts.

Crystal studies of several proteins permit the following inferences to be drawn. Some proteins can crystallize with grossly different complements of water, with the molecular skeletons of the interlocked $NC_{\alpha}C$ monomers remaining intact: however, it is only in the presence of a sufficient complement of water molecules that the proteins can achieve the orderly setting of the R-substituents of their monomers in the definite and specific spatial patterns in which their physico-chemical personality and so, also their functional potentialities, inhere. Physico-chemical studies indicate that many proteins have particle rather than molecular status, being dissociable into sub-units, which also have the protein character. That water can dissociate proteins is known from the case of horse hemoglobin which dissociates into pairs of sub-units simply on dilution. But the physico-chemical individualities of protein particles necessarily depend upon the inter- as well as the intra-sub-unit patterns of the atoms. Hence a second way in which water molecules can affect the activities of proteins is suggested.

Extrapolating from cases such as these, we can now visualize how water plays an essential role in biological function, as for example in the "re-activation" of the *Amoeba proteus* culture. Only by dissociating the dried mass of protein structures from one another are these structures able to reassume their physico-chemical individualities and perform their biological functions. The way in which a physico-chemical stimulus, the addition of water, is able to initiate vast changes is then seen to depend upon the fact that the activities of proteins depend upon their physico-chemical individualities not merely on their chemical structures. In "dry" insulin crystals, as in "wet" insulin crystals, definite structures, which we may identify with the skeletons of the molecules, remain unchanged. However, it is only in the "wet" crystals that any orderliness from unit cell to unit cell can be imputed to the R-group atoms; it is only in the "wet" crystal, therefore, that the protein structures achieve orderly arrangements of skeletons *and* substituents. After the same manner, we may visualize the proteins with intact chemical structures even in the "thin layer of greenish dust" and understand how the reactivation of the proteins, which underlies the reactivation of the culture, is effected by the water molecules, which enable the proteins to reassume their physico-chemical individualities.

---

[1] This work is supported by the Office of Naval Research under contract N8onr-579.

REPORTS ON LALOR FELLOWSHIP RESEARCH

Studies on the distribution of the oxidative pathway of glucose-6-phosphate utilization. Seymour S. Cohen.

Enzymes catalyzing the oxidation of glucose-6-phosphate and 6-phosphogluconate had been obtained from yeast, mammalian tissues, and *E. coli.* In recent studies in my laboratory it was shown that ribose-5-phosphate and triose phosphate was produced from 6-phosphogluconate in the enzymatic degradation of this substrate by cell-free preparations from yeast (*Science,* 1950, **111**: 543).

It has also been shown with *E. coli* that in adaptive growth on gluconate, the adaptive transphosphorylase, gluconokinase, is produced, converting gluconate to 6-phosphogluconate, used solely by the oxidative pathway. Nevertheless this partial bypass of the Meyerhof scheme permitted normal growth of the bacteria (*Nature,* In press).

It appeared of interest to see how widespread these dehydrogenases are from the point of view of assessing the general importance of this oxidative pathway, and secondly of determining whether the essential metabolite, ribose-5-phosphate, may be generated from other pathways. A spectrophotometric test was used for assaying homogenates of various organisms for the presence of dehydrogenases for glucose-6-phosphate and 6-phosphogluconate. The reduction of the coenzyme, triphosphopyridine nucleotide (TPN) was followed at 340 mμ as the reaction progressed in the absence of an autoxidizable carrier.

Both enzymes were found in homogenates of Chaetopterus sperm, the blue-green alga, *Tolypothrix lanata,* the red alga *Ceramium rubrum,* the green alga *Ulva lactuca,* and of frog heart.

Several attempts were made to demonstrate the enzymes in the protozoan, *Tetrahymena gelii,* and a brown alga, *Ascophyllum,* without success. The possible significance of negative results of this type will be discussed. However, the wide distribution of the enzymes is of interest.

Studies on spindle material. J. F. Danielli.

Experiments on the isolation of spindle material from the first division of the ovum of marine organisms were performed. After fertilization, the fertilization membranes are removed and the eggs allowed to swell to bursting in solutions having an osmotic pressure 10–30% that of sea water. Solutions containing mixtures of potassium and magnesium are preferable for this purpose. By this procedure, spindle material is obtained which is swollen and distorted.

Auxin effects in Bryopsis. Wm. P. Jacobs.

Although auxin, the plant growth-hormone, has been shown to be present in many algae, including *Bryopsis,* its role in algae is still unknown. Since the most striking effect of auxin in *flowering* plants is the induction of roots, experiments were designed to see if auxin would induce on the alga *Bryopsis* the algal equivalent of roots known as rhizoids.

Bryopsis plants were grown in a constant temperature bath ($21.5 \pm 1°$C.) in a diffusion gradient of indole-acetic acid, a synthetic auxin. The highest concentration of auxin was at the basal end of the plant. A striking difference was shown between the treated plants and the controls: the great majority of side-branches of plants in the auxin-gradient formed rhizoids at their bases, while no rhizoids were formed by any of the side-branches of any of the control plants.

Three replications of the experiment gave similar results.

A further effect of the auxin-gradient was found. Inverted *Bryopsis* plants with the highest auxin concentration at the original base showed nullification or reversal of the normal tropisms. The rhizoids, which are normally positively geotropic, grew away from the pull of gravity and toward the higher auxin concentration. The side-branches, positively phototropic in the controls, continued growing at a 45° downward angle in the treated plants. These changed tropiotic reactions are in agreement with the hypothesis that tropisms in *Bryopsis* are, like those of flowering plants, controlled by differential distribution and sensitivity to auxin.

A number of regeneration experiments were also tried with successful results.

The results relating to rhizoid formation and tropisms provide the first evidence that the ubiquitously occurring auxin has very similar effects in both algae and flowering plants.

Respiration and iodine uptake by the brown algae. SALLY KELLY.

The brown algae are noted for their capacity to accumulate iodine from dilute amounts in sea water. In experiments designed to demonstrate whether this property resembles that of salt accumulation by cells of higher plants, in being governed by respiratory mechanisms, the respiration of the brown algae, in particular *Ascophyllum nodosum* (Linnaeus) Le Jolis, and its effect on the uptake of radioactive iodine from sea water were examined.

The age of the tissue, thickness of segments and pH of the medium were among the factors which influenced the endogenous respiratory rate. Among the compounds which stimulated respiration were glucose (0.001 M), sucrose (0.01 to 0.05 M), malic, fumaric and succinic acids (0.025 M) and pyruvic acid (0.0025 M). Fermentation also was increased in the presence of glucose, pyruvic acid and malic acid. Respiration was inhibited by potassium cyanide (0.0001 M), sodium azide (0.001 M), iodoacetic acid (0.01 M), malonic acid (0.005 M), and potassium fluoride (0.025 M). Mannitol, a storage product of the brown algae, and catechol, had no stimulating effects. The inhibitors, fluoride and malonic acid, and the organic acid substrates were ineffective at the pH of the control medium, 6.5; their effects were observed, however, when the pH was lowered to 4.5, suggesting that the penetration of these compounds is an important factor in determining their influence on respiration.

The uptake of potassium iodide in concentrations of 0.05 to 0.1 mg./l (the concentration of iodine in sea water), tagged with the radioactive isotope, I^{131}, was influenced by such factors as age of the tissue and pH of the medium. The rate of uptake during the first hour of exposure to the radioactive solutions was rapid and fell off gradually during succeeding hours. The uptake by the algae during the experimental periods was accompanied by a corresponding decrease in the radioactivity of the solutions. Of the compounds which influenced respiration, glucose and sucrose (0.025 M) increased the amount of radioactive iodine taken up. Effective inhibitors were malonic acid (0.025 M), sodium azide (0.0001 M), idoacetic acid (0.0001 M) and potassium cyanide (0.005 M).

These results suggest that the respiratory mechanisms of the brown algae resemble those known for higher plant cells and that the phenomenon of iodine uptake by the algae requires energy of respiration. Whether the energy source comes chiefly from glycolytic or from oxidative processes, however, will require further study.

The effect of divalent ions on Echinoderm phosphatases. C. ALBERT KIND.

Tissue homogenates of many marine invertebrates have been shown to exhibit glycerophosphatase activity. With the exception of the Porifera, which appear to possess only an acid phosphatase, the pH-activity curves of these preparations show maxima at pH 4.5–5.0 and 8.0–8.5 and thus resemble the vertebrate phosphatases in this respect. In the interest of demonstrating an even closer relationship between the invertebrate and vertebrate enzymes a preliminary investigation has been made of the effects of magnesium, manganese, and cobalt ions on the alkaline phosphatases of the brittle-star *Ophioderma brevispinum* and the sea cucumber, *Thyone briareus.* The ability of these ions to activate the alkaline phosphatases of vertebrate tissues is well known. Recent interest in beryllium as an inhibitor of alkaline phosphatase suggested investigation of the effect of beryllium on the echinoderm enzymes.

The enzyme preparations used in the present study consisted of 4% homogenates of the central disk of *Ophioderma* and of the digestive tract of *Thyone.* Incubation with β-glycerophosphate was at 25°C. for 24 hours at pH 8.6. The metals were added as the chlorides and the concentrations given represent concentration in the incubation mixture.

Beryllium reduces phosphatase activity of both preparations in concentration as low as 10^{-6}M. At a concentration of 10^{-4}M the inhibition is about 40% and complete inhibition occurs at 10^{-3}M. Beryllium inhibition at 10^{-4}M is antagonized by the addition of either 10^{-4}M manganese or 10^{-4}M cobalt, but magnesium is without effect. This antagonism of beryllium inhibition is similar to that described by Dubois *et al.* (*Science*, 1949, **110**: 420) for rat serum phosphatase.

The dialyzed homogenates can be activated by the addition of magnesium, manganese, or cobalt; maximum activation being obtained at concentrations of 10^{-4}M, 10^{-5}M, and 10^{-5}M, respectively.

In general the effects of divalent cations on the alkaline phosphatases of these two echinoderms resemble very closely the effects on vertebrate phosphatase. These observations suggest that the mechanisms involved in these effects are also comparable.

Reactions related to photosynthesis and respiration. ALAN H. MEHLER.

Since Hill demonstrated that isolated chloroplasts could utilize light energy to reduce certain iron salts and liberate molecular oxygen, there have been many investigations into the nature of this reaction as a part of the mechanism of photosynthesis. The ability of spinach chloroplasts to react with a variety of oxidants had been explored previously. In order to determine whether the pattern obtained was characteristic of all chloroplasts, attempts were made to obtain active cell-free preparations from different plants. No sustained activity was found in chloroplast preparations from the green algae *Vaucheria, Nitella, Cladophora*, and *Ulva*, from two species of *Equisetum*, or from duckweed, *Lemna minor*. An extract of *Tolypothrix*, a blue-green alga, evolved some gas when illuminated with quinone, and experiments are in progress to isolate the active component.

Clendenning recently found that *Chlorella* acts like chloroplasts in evolving oxygen when illuminated in the presence of Hill oxidants, and that the ability to carry out photosynthesis and respiration is irreversibly lost in the process. The effect of quinone is markedly different with different algae. *Ulva*, like *Chlorella*, carries out a typical Hill reaction, and has both respiration and photosynthesis completely inhibited. With *Tolypothrix* there is an incomplete Hill reaction, photosynthesis is inhibited, but respiration is not affected. The photosynthesis of *Dasya*, a red alga, is inhibited by quinone, but no Hill reaction is seen. Instead, the effect of light is to increase the rate of respiration, and the increase is maintained in the dark, similar to the increase obtained in respiration of cell-free preparations of spinach.

The identification of a typical oxidative enzyme, malic dehydrogenase, in extracts of *Ceramium rubrum, Ulva*, and *Tolypothrix* indicates that these various algae probably use a respiratory mechanism very similar to those of animals and other aerobic organisms. In the first two species isocitric dehydrogenase was also demonstrated.

Separation and characterization of neurosecretory material from Fundulus heteroclitus. STUART W. SMITH.

Scharrer and others have established the cytologically based concept of secretory activity of certain hypothalamic nerve cells in many, but not all, vertebrates examined. It is believed that the neurosecretory colloid is transported along the axons of these cells to the *pars nervosa hypophysis.* Melville and Hare demonstrated that the supraoptic region contains extractable materials with antidiuretic activity. On the basis of unpublished cytological evidence from material stained by the chrome alum-hematoxylin technique, shown by Bargmann to be specific for neurosecretory material, I am led to believe that hypothalamic neurosecretory material is probably uniformly present in all vertebrates, and that it is probably identical with the *pars nervosa* hormone(s). However, no one has thus far reported direct chemical or physiological evidence to prove the latter thesis.

It was the purpose of this work to separate morphologically intact neurosecretory material from hypothalami of *Fundulus heteroclitus* by fractional centrifugation, and to test the separated material chemically and physiologically. For each methodological trial 25–50 hypothalami, carefully freed of hypophysial tissue, were obtained from decapitated male animals and chilled to 0–4°C. Two homogenizing methods and many suspending media (Kassel's, Kallman's, and Van't Hoff's solutions; 0.88, 2.00, and 2.50 *M* sucrose, and 1.5 *M* NaCl) as well as many combinations of centrifugal force and time were tried. Temperature during centrifugation was 0–5°C. Results were controlled by examination of smeared centrifugates stained with chrome alum-hematoxylin-phloxin.

At the time of writing this report, it has not been possible to obtain an essentially pure preparation of neurosecretory droplets. However, concentration of intact droplets by a factor of approximately 100 has been achieved. The nucleoproteins of persistently contaminating nuclei have been eliminated by homogenization in 10 volumes of 1.5 M NaCl followed by storage in the cold for 12–16 hours prior to centrifugation.

Studies of effects of the concentrates on blood pressure of Fundulus have not been technically possible thus far. Antidiuretic activity in euryhaline teleosts is studied only with the greatest difficulty. A large supply of frozen, concentrated neurosecretion is being collected, therefore, to be used later in testing for pressor effects in a larger teleost and water balance activity in toads, as well as for attempts at further purification.

During the course of the work described, I have also collected fixed material from marine vertebrates to supplement a cytological study of neurosecretory phenomena in the sympathetic ganglion cells of higher vertebrates.

Vol. 99, No. 3 December, 1950

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

SOME OBSERVATIONS ON A LUMINESCENT FRESHWATER LIMPET FROM NEW ZEALAND

BERNARD J. BOWDEN

University of Otago, Dunedin, New Zealand

In view of the statement of Harvey (1940) that ". . . luminous animals are practically entirely marine or terrestrial. No examples of freshwater luminous organisms are known except bacteria and an aquatic glowworm . . .," the luminous properties of the New Zealand freshwater mollusc, *Latia neritoides,* would appear to be a unique and surprising phenomenon. This, together with the fact that, apart from the cephalopods, only three luminescent species of molluscs have been hitherto recorded (Harvey, 1940) is sufficient justification for a full investigation of the apparently exceptional properties of this freshwater limpet.

Latia is a monotypic genus restricted to the North Island of New Zealand. It belongs to the family Ancylidae in which no other luminescent forms have been recorded. The two other New Zealand species (*Gundlachia neozelanica* and *G. lucasi*) are certainly not luminescent.

The luminescence of Latia was first recorded by Suter in 1890 and again referred to in his comprehensive Manual of New Zealand Mollusca (1913). Although the phenomenon is well known to many New Zealanders, its nature and mechanism have never been studied.

It was with this end in view, therefore, particularly in the light of modern researches in the field of bioluminescence, that the present investigation has been undertaken.

MATERIAL AND METHODS

Specimens of *Latia neritoides* were collected near Auckland in December, 1949, and were immediately placed in Bouin's fluid. Subsequently during the investigation, living specimens were obtained in May, 1950, for experimental and histological purposes. It was found that these animals remained alive in the laboratory for more than three weeks if placed in brown glass bottles in the dark, whereas if kept in white glass bottles and exposed to the light they died within a day or two.

In order to contrast the histological structure of Latia with that of an allied nonluminescent form, specimens of *Gundlachia lucasi* were collected locally and submitted to simliar histological procedures.

Luciferin-luciferase reaction. Three animals were shaken in 10 ml. of water in a test-tube at room temperature for 10 minutes. The solution was then decanted into another test-tube and allowed to stand until all luminescence had disappeared, leaving a solution of the thermolabile luciferase. Three more animals were placed in another test-tube with 10 ml. of water at 70° C., crushed, kept at that temperature in a water bath for 10 minutes and then allowed to cool. At 70° C. the thermolabile luciferase is denatured and the substrate, luciferin, is thus obtained unoxidised in solution.

Five ml. of clear solution were then decanted from each tube, neither of which at this stage showed any luminescence. Upon mixing the two samples, however, a brilliant pale green light was produced. This indicated a positive luciferin-luciferase reaction.

The possibility that the presence of "cytolytic" substances in the hot-water solution might cause luminescence, by breaking down residual granules of luciferin in the cold-water extract, was disproved by the method described by Harvey (1940) of adding to the cold water solution several drops of ether which produces a similar effect. No luminescence resulted in this solution, thus proving the absence of residual granules of luciferin, while the presence of the ether did not inhibit the production of light when the two solutions were mixed. This appeared to confirm the conclusion that the reaction is a true luciferin-luciferase oxidation.

Histology. For histological study the animals were fixed in Bouin's fluid. They were embedded in paraffin and sectioned at 10 μ, both longitudinally and transversely. A variety of staining procedures were used: haematoxylin and eosin, Mayer's mucicarmine, and the Azan stain. An attempt was also made to study the innervation of the specific cells by means of the methylene blue technique, but this was unsuccessful and consideration of this aspect of the problem is deferred.

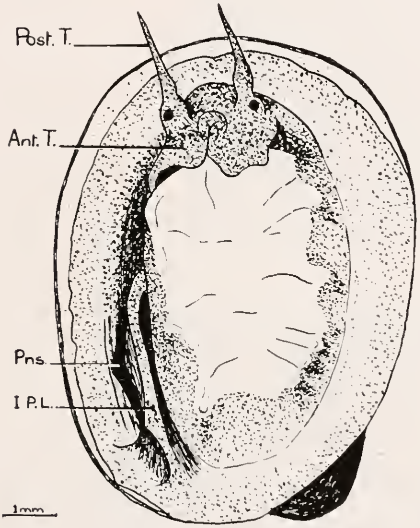
In order to determine the actual source of the luminescent material, several specimens were submitted to prolonged stimulation, by rubbing with a seeker the luminescent zones, in order to exhaust the specific cells. These animals were then submitted to the same histological procedures as before (Figs. 4, 5).

OBSERVATIONS

General. Latia is common in clear streams, often in rapids and other situations where the current is swift; living on the sides or undersurfaces of clean boulders, its limpet shape offers little resistance to the smooth flow of water. It is also found, but much less commonly, in lakes in places where there are clean rocks and considerable water movement, usually very close to the shore. An analysis of the mixed supply of water from the Waitakere Ranges near Auckland indicates its low salt content: sodium chloride was present as 3.04 parts and magnesium chloride as 0.76 parts per 100,000. Latia is abundant in all of these streams and from one of them the specimens used in this investigation were collected.

Like most other luminous forms, Latia shuns strong light and, as already stated, it soon dies if kept exposed to light. It has a well developed eye with the "pupil" directed forwards, and appears to be well adapted to perceive the direction of incident light.

Latia commonly reaches a length of 8.5 mm., a breadth of 6 mm. and a height of 3 mm. Its shell has a thin calcareous layer covered by a stout dark brown periostracum, smooth except for growth lines. Posteriorly, there projects forwards (horizontally within the shell) a semicircular shelf or lamella which on the right side is prolonged forwards into a free calcareous lingula (Fig. 2) which in the living state supports the medial wall of the pneumostome (the inferior pallial lobe) (Fig. 1). Both the lamella and the lingula give origin to the muscular mass of the foot (Fig. 3) and so fix the shell very firmly to the body. Although probably a great advantage in a swift stream, this nevertheless greatly restricts the mobility of the animal relative to the shell, for when placed on its back it is unable to right itself; Gundlachia, which lacks these processes, is able to perform the manoeuvre easily. The mantle cavity of Latia, like that of some marine Basommatophora (the Siphonariidae, the Gadiniidae), is water filled, but as in the Gadiniidae does not contain a branchia. The skeletal support afforded by the lingula appears to prevent both the closure and the over-dilatation of the pneumostome while directing a stream of water into the mantle cavity to flush it.



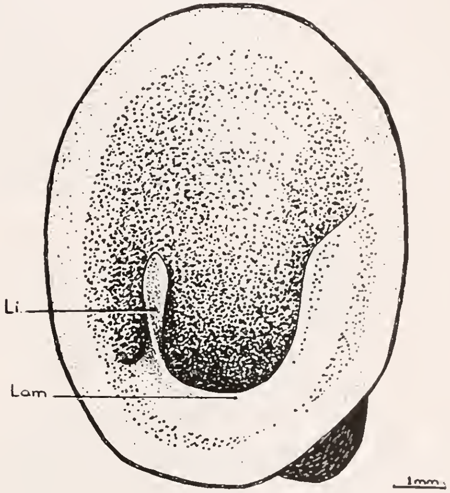


FIGURE 1. *Latia neritoides* Gray. Ventral view of the intact animal. All the parts of the animal that are shown are light producing, except the sole of the foot, the posterior tentacles, and the interior of the pulmonary cavity. Pns., pneumostome. I.P.L., inferior pallial lobe. Ant. T., anterior tentacle, Post. T., posterior tentacle.

FIGURE 2. The interior of the shell showing the semicircular lamella (Lam.) and the lingula (Li.)

Reference may be made to some remarks on the anatomy of Latia by Hutton (1881). Probably because of poorly preserved material he stated that the tentacles were transversely ringed and that the eyes were lateral to the bases of the tentacles. This is obviously not the case either in fresh or fixed specimens (Fig. 1). Hutton also gave the radular formula as 27 + 1 + 27 but this is certainly wrong, since the number of laterals is approximately 110.

Latia, like the rest of its sub-order, is a vegetarian. The gut is usually filled with diatoms and what appear to be the remains of algae. Similar diatoms are found on the surface of the shell.

The luminescence. Whenever the animal is disturbed, as by shaking in a vessel or tapping the shell, luminous mucus appears in all parts of the groove between the foot and the mantle. This is visible even in diffused daylight as a greenish glow, and in the dark the effect is much more striking. The animals have never been seen to luminesce spontaneously at night either in the laboratory or in their natural habitat, but when they are stimulated they produce the glow equally well during the day or night. Where the mantle groove is touched with a seeker, that region in particular glows most strongly. Shaking the animal in a test tube or rubbing it, foot down, across a glass slide produces trails of glowing mucus from which the light slowly fades.

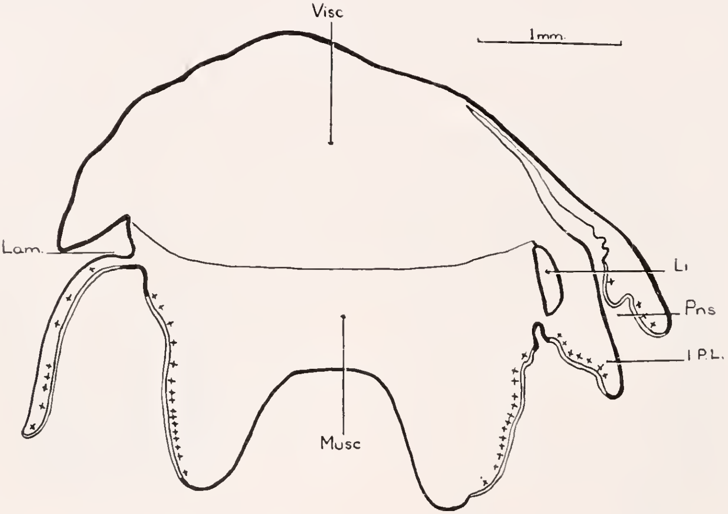
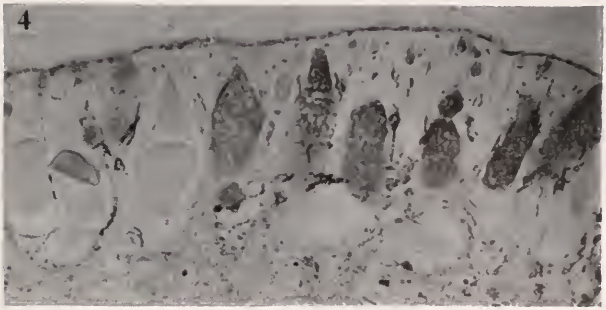
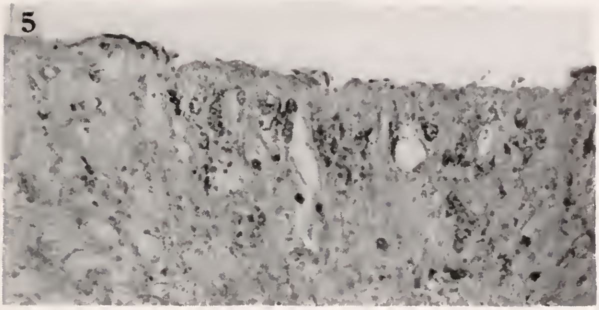


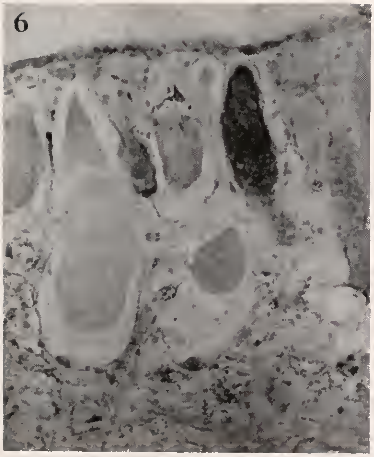
FIGURE 3. Outline of a transverse section (camera lucida). The crosses show the approximate number and distribution of granular cells. (These represent the average distribution in six non-consecutive sections from the same specimen.) Musc., muscular mass of the foot. Visc., visceral hump. (Other letters as for Figs. 1 and 2.)

Clearly then the phenomenon is an extracellular one, and experiments to determine the nature of the luminescence show that it depends on the action of luciferase upon the substrate luciferin, which appear to be produced together from specific areas, particularly along the walls of the mantle groove.

Histology. Examination of the sectioned animal shows that certain parts of its surface present a very striking and characteristic appearance. These regions are the surface of the head, the anterior tentacles, the lateral surfaces of the foot, the inferior pallial lobe, and the free surface of the mantle (but not of the pulmonary cavity). In all these situations, just beneath the simple cuboidal surface epithelium and lying in the loose subepithelial connective tissue, are large numbers of intermingled mucous cells and granular cells, together with branching melanophores and muscle fibres (Figs. 4, 6, 7). Since these regions correspond precisely to those from which the maximum luminous response may be obtained experimentally, they obviously represent the structural apparatus responsible for the luminescence.







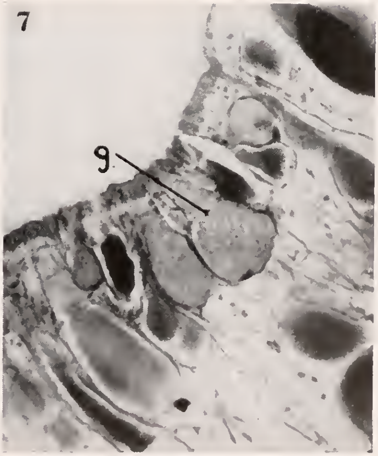


FIGURE 4. General view of luminous epithelium (Haematoxylin and Eosin) × 180.

FIGURE 5. A similar view after the animal had been stimulated to exhaustion (Haematoxylin and Eosin).

FIGURE 6. Two mucous cells in this figure show basally placed nuclei and the large size of the vacuole (Haematoxylin and Eosin) × 250.

FIGURE 7. The granular cell (g) in the center of the figure shows a flattened, basally situated, darkly stained nucleus and granules packing the whole cytoplasm (Azan).

The mucous and granular cells appear to be most probably the specific elements concerned in the phenomenon, since they alone are absent from the non-luminous areas, and they are not found in other non-luminous mollusca including the related Gundlachia.

1. *Mucous cells.* These are large elongated ovoid cells which may reach a length of 200 μ and a diameter of 40 μ. They taper markedly as they approach the surface epithelium and in many cases their secretion could be observed being extruded at the surface from the apex of the cell. The cells present large swollen vacuoles containing mucus, which stains pink or pinkish-purple with haematoxylin and eosin, purple with Azan, but is not stained by Mayer's mucicarmine. The cytoplasm, which is greatly attenuated, stains blue with Azan but is indistinctly stained with eosin. The nuclei of these distended cells are always basally situated and somewhat compressed.

2. *Granular cells.* These are elongated cells, rather smaller than the mucous cells (150 μ × 30 μ) but like them project into the epidermis. Their cytoplasm is packed with densely basophilic granules which in haematoxylin preparations generally obscure the nucleus. Where it is visible the nucleus is generally, but not always, basally situated and very flattened. With Azan the nuclei stain red, the granules blue.

3. *Changes after stimulation.* Instead of the large and distinctive mucous and granular cells, which were previously such a striking feature of the subepithelial tissue, it is now difficult to identify any spechic cells at all. While empty mucous cells, now slender and devoid of secretion, are still recognisable here and there, it is practically impossible to identify with certainty the previously granular cells. There is, in fact, a strong possibility that not only are the entire contents of the granular cells discharged but that the nucleus is extruded as well, in which case mucous cells alone would remain in the exhausted luminescent areas. Whether or not this applies to all the granular cells, it is certainly the case that smears of mucus do contain nuclei of an appearance closely similar to those of the granular cells.

Discussion

The above observations establish quite clearly that Latia is a completely aquatic gasteropod with marked adaptations to life in strong currents; and that its bright luminescence is extracellular and is due to the secretion of luciferin and luciferase.

Harvey (1940) has stated that in only five of the very numerous orders of plants and animals in which luminescence occurs, can the luciferin-luciferase reaction be demonstrated *in vitro*. These include beetles (fireflies), some ostracods, a few polychaete worms, one squid, and now Latia joins *Pholas dactylus* as the second example in the lower mollusca (Plocamopherus and Phyllirrhoë have not yet been tested). Since the only other known example of fresh water luminous animals are the aquatic glowworms, it is interesting to compare Latia with these animals. These glowworms are actually larval coleoptera and have been recorded only from Asia. Annandale (1900) has described one (? Luciola sp.) from Calcutta; another (identified provisionally as *Pyrophanes similis*) was found on the Island of Celebes and described by Blair (1927); while two others from Japan (*Luciola cruciata* and *L. lateralis*) have been described by Okada (1928). Since in these forms, which all possess tracheal gills, the tracheae are full of air, the functioning of the luminescent organs is presumably the same as that in the adults which are terrestrial fireflies. In these, in striking contrast to Latia, the production of light is intracellular and the luminous organs are covered like the rest of the body by a chitinous cuticle (Buck, 1948; Okada, 1928).

The luminous apparatus of Latia is similar to that of certain other luminous invertebrates (Dahlgren, 1915–17), particularly those with a naked epidermis or with only a thin cuticle, such as the coelenterates, polychaetes (especially Chaetopterus), and enteropneusta (Ptychodera). It bears, however, by far the closest resemblance to the luminous apparatus of another mollusc—the peculiar, pelagic opisthobranch *Phyllirrhoë bucephala*, in which both the mucous and the granular cells are almost identical with those of Latia; although I have not observed in Latia the prominent nerve-endings which are described by Dahlgren (1916a) in relation with the granular cells of Phyllirrhoë. In view, however, of the doubt cast on the numerous (and extremely conflicting) descriptions of light-cell innervation in the fireflies (Buck, 1948), the problem of whether or not there is a direct innervation of these cells in mollusca must remain open until such nervous connections have been demonstrated by recognised neurological techniques.

The possibility mentioned above, that the secretion of the granular cells in Latia may be holocrine, seems to be supported by some statements of Dahlgren. He suggests, for instance, that in Chaetopterus the granular cells may be "of a secretory type that are destroyed by one cycle of secretory activity" (1916b); again, when dealing with Pholas (1916a), he recalls Forster's observation that the granules in the granular cells of Pholas fill the cell and appear to be in contact with the nucleus. He comments that this is unusual except in cells which die on discharge of their contents but the mucus does not seem to have been examined for nuclei.

We see, therefore, that although Latia is quite unique among freshwater animals, the actual mechanism of its light-production closely resembles that of certain other marine luminous species, and thus does not bring us any closer to the solution of the very interesting problem: why is it that luminous forms, so common in the sea are so rare in fresh water? It is, however, possible to say now that the reason for their rarity cannot simply depend on a low concentration of salt.

Of the functions which may be attributed to the luminescence of Latia, no positive conclusions can be drawn. It has been suggested that luminescence might subserve three principal purposes not necessarily mutually exclusive: a lure for food; a sexual recognition signal; or a defensive mechanism. In the case of Latia, the first may be dismissed because the animal is a typical herbivore. The second, although it cannot be completely discounted, is unlikely for two reasons: Latia where it occurs is a common species, it is moreover an hermaphrodite and it would seem to have fewer rather than more difficulties to overcome than the nonluminous operculates which occur with it; furthermore its light production is not seasonal and is readily elicited by harmful stimuli.

As for the third possibility: what little evidence there is on the food habits of the fresh water fauna of New Zealand indicates that Latia is eaten by both trout (Phillips, 1929) and eels (Cairns, 1944) but detailed statistics are not available. It seems reasonable to conclude, however, that it is not unpalatable so that the luminescence cannot be equivalent to a warning coloration. A more obscure defensive action has been suggested for other luminous forms by Burkenroad (1943) who has put forward the view that light from specimens attacked by predators might attract secondary predators for the purpose of driving off the primary ones. However the problem is obviously one which cannot be solved until more detailed field work can be undertaken.

In conclusion, it may be stated that the luminescence cannot be regarded as physiological accident or metabolic by-product. The large size and high degree of specialization of the specific cells are themselves sufficient evidence to the contrary. Latia, of course, is not exceptional in lacking an obvious use for its light, for luminous species in which a function is known are the exception rather than the rule (Harvey, 1948).

ACKNOWLEDGMENTS

I wish to express my gratitude to Professor W. E. Adams for allowing me to use the laboratories of this Department and for criticising the manuscript; to Mr. A. D. Mead, City Waterworks Engineer, Auckland, for providing the water analysis figures; and to my parents for collecting specimens and sending them to Dunedin.

SUMMARY

1. *Latia neritoides* Gray is a pulmonate freshwater limpet from New Zealand, and is highly adapted to life in streams.

2. It is brilliantly self-luminous due to the extracellular secretion of luciferin and luciferase, and is therefore unique among freshwater animals.

3. The luminous epithelium occurs over much of the animal and is similar in appearance to that found in many marine invertebrates. It is especially similar to that of another gasteropod, Phyllirrhoë. In both species similar mucous and granular cells are found lying in the subepidermal connective tissue and projecting into the epidermis.

4. No definite function can yet be assigned to the light.

LITERATURE CITED

ANNANDALE, N., 1900. Insect luminosity (An aquatic lampyrid larva). *Proc. Zool. Soc. London,* p. 862.

BLAIR, K. G., 1927. An aquatic glowworm. *Nat. Hist. Mag.,* **1**: 59.

BUCK, J. B., 1948. The anatomy and physiology of the light organ in fireflies. *Ann. N. Y. Acad. Sci.,* **49** (3): 397–482.

BURKENROAD, M. D., 1943. A possible function of bioluminescence. *J. Marine Res.,* **5** (2): 161–164.

CAIRNS, D., 1944. Eels, life history of the two species of N. Z. freshwater eel. Wellington Acclimatisation Society, N. Z.

DAHLGREN, U., 1915–1917. The production of light by animals (A series of papers). *J. Frank. Inst.,* **180**: 513–537, 717–727; **181**: 109–125, 243–261, 377–400, 525–556, 659–699, 805–843; **183**: 79–94, 211–220, 323–348, 593–624, 735–754.

DAHLGREN, U., 1916a. The production of light by animals. Molluscs. *J. Frank. Inst.,* **181**: 377–400.

DAHLGREN, U., 1916b. The production of light by animals. Polychaetes. *J. Frank. Inst.,* **181**: 659–699.

HARVEY, E. N., 1940. Living light. Princeton University Press.

HARVEY, E. N., 1948. A general survey of bioluminescence. *Ann. N. Y. Acad. Sci.,* **49** (3): 329–336.

HUTTON, F. W., 1881. Notes on some pulmonate mollusca. *Trans. N. Z. Inst.,* **14**: 150–157.

OKADA, Yø. K., 1928. Two japanese aquatic glowworms. *Trans. Entom. Soc. London,* **74**: 101–107.

PHILLIPS, J. S., 1929. A report on the food of trout. *N. Z. Marine Dept., Fisheries Bull.,* No. 2.

SUTER, H., 1890. Miscellaneous communications on N. Z. land and freshwater molluscs. *Trans. N. Z. Inst.,* **23**: 93–96.

SUTER, H., 1913. Manual of the New Zealand mollusca. N. Z. Government Printer.

CHLORIDE EXCRETION IN THE HEAD REGION OF FUNDULUS HETEROCLITUS

JEAN BURNS AND D. EUGENE COPELAND

Arnold Biological Laboratory, Brown University, Providence, Rhode Island

The ability of marine teleosts to actively excrete salts from the head region was first demonstrated by Smith (1930). Although cautious in his statement, he assumed the function to be localized in the gill structure. The perfusion experiments of Keys (1931), Bateman and Keys (1932) and Schlieper (1933) proved that the gill did indeed have that function.

The identity of the cell responsible for the chloride transfer was first suggested by Keys and Willmer (1932). They gave no proof of its function, but made deductions to that end. Bevelander (1935 and 1936), after a comparative survey of many fish gills, was inclined to the view that the general respiratory epithelium, rather than any special cells, was responsible.

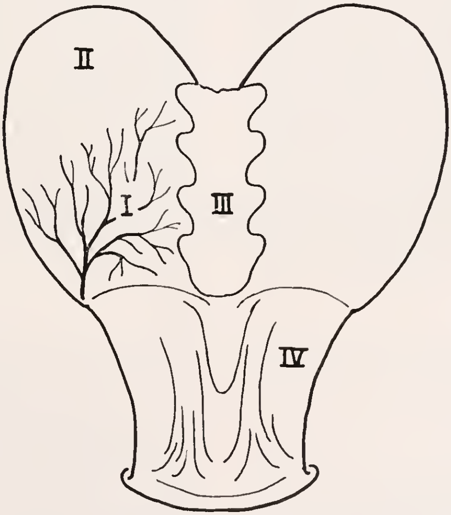


FIGURE 1. A diagram of the lower jaw with the gills removed and the opercula spread out. The normal, gross vascular supply of the operculum is shown on one side (I). The rest of the operculum (II) is relatively poorly supplied. Other areas investigated are the region of gill attachment (III) and the lateral floor of the mouth (IV).

The first significant experimental evidence was furnished by Liu (1942) who found that a certain cell type in the fresh water paradise fish, *Macropodus opercularis,* hypertrophied when the fish was exposed to gradually increased salinities. Later, Copeland (1948) gave experimental evidence that in the euryhaline *Fundulus heteroclitus* the cell homologous to those described by Keys and Willmer (1932) and Liu (1942) is the cell responsible for chloride excretion in the gill. It has also been suggested (Pettengill and Copeland, 1948) that the same cell can reverse its polarity and be responsible for the chloride absorption observed in fresh water conditions (Krogh, 1937).

Since the chloride excreting cell of *Fundulus heteroclitus* has been investigated and identified by a number of techniques, it was decided to apply a number of them to the rest of the epithelium of the head cavities, both oral and pharyngeal, to see if the special cell is confined to the gill.

MATERIALS AND METHODS

The fish used for this study were adult *Fundulus heteroclitus* which had been fully adapted to sea water.

They were killed by decapitation. The lower jaw and opercula were separated from the rest of the head and pinned down. Samples of epithelium from the numbered areas of Figure 1 were stripped from the underlying connective tissue and fixed.

Osmiophilia preparations were made following the directions of Ludford (1926).

The mitochondria were demonstrated by the method outlined by Copeland (1948) with the substitution of the following for his picric acid differentiation.

1. Differentiate with 1% methyl green, 5 sec.
2. Wash in distilled water, 10 sec.
3. Rinse in 95% alcohol, 5 sec.
4. Place in absolute alcohol I, 10 sec.
5. Finish dehydration in absolute alcohol II, 3–4 min.
6. Clear in toluene, mount in clarite.

Some of the tissue was fixed in cold 1 : 1 acetone-absolute alcohol and 7 micron sections of this material were incubated at 37° C. in 0.4% Ca glycerophosphate and handled according to Gomori's (1939) directions for the demonstration of alkaline phosphatase.

RESULTS

Chloride cells are found distributed throughout the epithelium of the head cavity, the distribution of the cells showing a distinct correlation with the vascularity of the tissue.

EXPLANATION OF FIGURES

FIGURE 2. Preparation by the Ludford technique to show osmiophilia.

FIGURE 3. Alkaline phosphatase preparation by the Gomori technique.

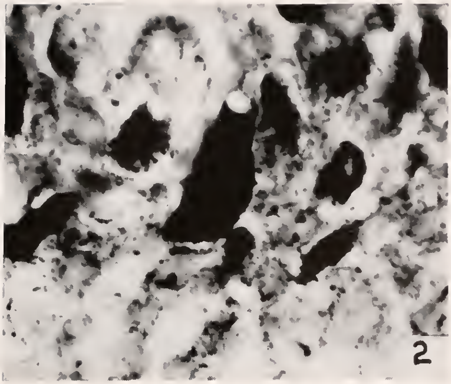
FIGURE 4. Mitochondrial preparation by the Regaud-Altmann acid fuchsin technique.

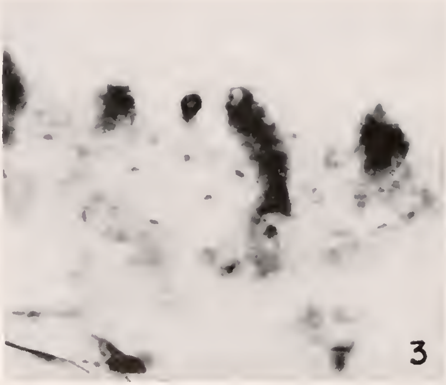
FIGURE 5. Epithelium from a relatively avascular region (area II of Fig. 1). No chloride cells visible in this particular view, though they are found sparsely scattered in the region. The granular cells are eosinophil granulocytes. Mitochondrial preparation.

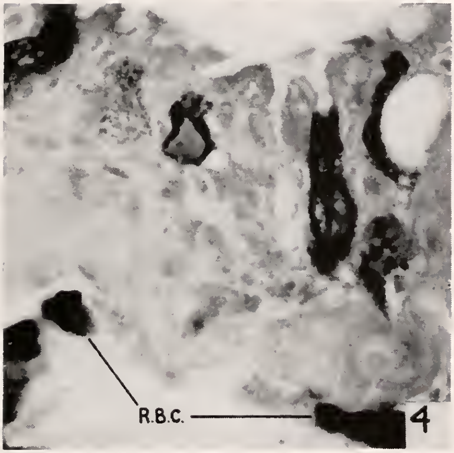
FIGURE 6. Epithelium from a well vascularized region (area I). Note the number of chloride cells and the proximity of the circulation. Mitochondrial preparation.

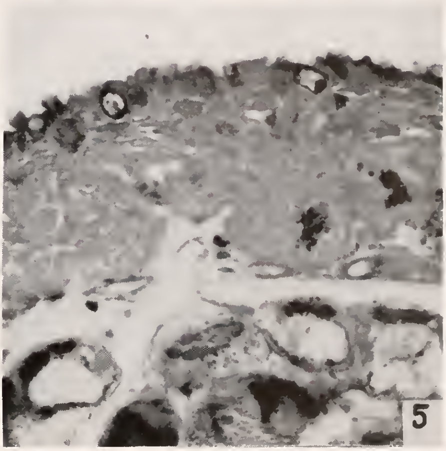
FIGURE 7. Epithelium from the same area as Figure 5, but from an animal that had an abnormal degree of vascularization in that area. An accompanying density of chloride cells is found. Mitochondrial preparation.

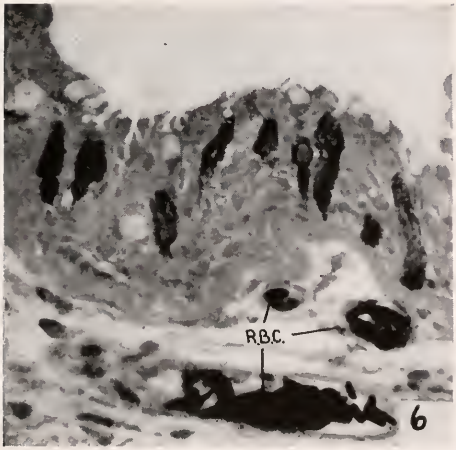
Magnification: Figures 2, 3, 4 approximately 800 × (oil immersion) and 5, 6, 7 approximately 400 × (high dry).

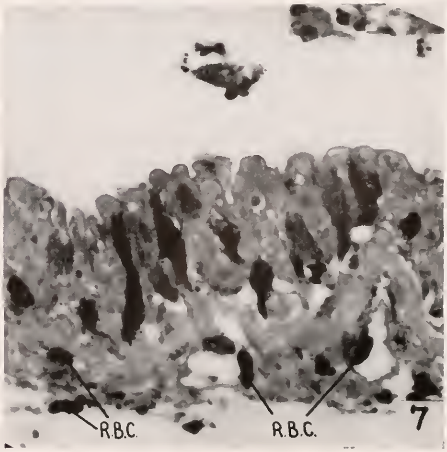












Figures 2–7

The morphology of the cells is like that of the chloride cells of the gills. The osmiophilic (Fig. 2) and alkaline phosphatase (Fig. 3) pictures correspond to those shown of the gill chloride cells by Copeland (1950) and Pettingill and Copeland (1948). The mitochondria pattern (Fig. 4) is much like that in the gills (Copeland, 1948). One dissimilarity can be noted: the cells of the gills usually contain a greater density of mitochondria.

Chloride cells are found in all parts of the cavity. In areas II, III, and IV of Figure 1 and in the roof of the mouth they are sparsely distributed, histological preparations showing many sections devoid of cells (Fig. 5). In area I they are almost as numerous as in the gill epithelium (Fig. 6).

The correlation between number of chloride cells and degree of vascularity of the epithelium is very marked. In an area of good vascularity the cells are always present in abundance (area I). The reverse is seen in regions of poor vascularity (areas II, III, IV and the roof of the mouth). That a relation exists between the cell population and vascularity of the tissue is indicated by the following observation. In one case the grossly visible blood supply extended to an unusual degree into a normally "avascular" region (area II). Histological examination revealed a large number of cells where normally few are found (Fig. 7).

DISCUSSION

The chloride excreting mechanism in the head region of teleosts has a much wider basis than originally assumed. Chloride excreting cells similar to those in the gill filaments are found in the rest of the oral, pharyngeal, and opercular epithelium. The number in the oral cavity and in the region of the gill bar attachment (pharnygeal) is probably not great enough to play a significant part in osmoregulation. However, the relatively huge number in the opercular lining is significant.

It is very interesting that there is a correlation between the number of cells and the degree of vascularity seen in the operculum. Since the cells presumably control the salt level of the body through the mediation of the circulatory system, it is significant that such a correlation is found.

Keys and Willmer (1932) calculate that in a 250 gram eel there are 3 to 6 million chloride cells present in the gill, enough to account for the observed salt excretion. Assuming the two teleosts to be homologous in this basic arrangement, the discovery of additional cells increases the adequacy of the tissue as a mechanism for extra-renal osmoregulation.

SUMMARY

1. The chloride excreting cell is not limited to the gill epithelium, being found in other regions of the head, especially the inner surface of the operculum.

2. With one exception, the cells appear identical in morphology and in response to Regand-Altmann, Ludford, and alkaline phosphatase techniques. The exception is that the mitochondria are usually more densely packed in the branchial cells.

3. In the operculum, the population density of the chloride cells is in positive ratio to the vascularity of the tissue. Such a topographical positioning of the cell is significant to its function of removing chlorides from the circulatory system.

LITERATURE CITED

BATEMAN, J. B. AND A. KEYS, 1932. Chloride and vapour-pressure relations in the secretory activity of the gills of the eel. *J. Physiol.*, **75**: 226–240.

BEVELANDER, GERRIT, 1935. A comparative study of the branchial epithelium in fishes, with special reference to extra-renal excretion. *J. Morph.*, **57**: 335–352.

BEVELANDER, GERRIT, 1936. Branchial glands in fishes. *J. Morph.*, **59**: 215–224.

COPELAND, D. EUGENE, 1948. The cytological basis of chloride transfer in the gills of *Fundulus heteroclitus*. *J. Morph.*, **82**: 201–227.

COPELAND, D. EUGENE, 1950. The adaptive behavior of the chloride cell in the gill of *Fundulus heteroclitus*. *J. Morph.* (in press).

GOMORI, G., 1939. Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. Exptl. Biol. Med.*, **42**: 23–26.

KEYS, ANCEL B., 1931. Chloride and water secretion and absorption by the gills of the eel. *Zeit. vergl. Physiol.*, **15**: 364–388.

KEYS, ANCEL B. AND E. N. WILLMER, 1932. "Chloride secreting cells" in the gills of fishes, with special reference to the common eel. *J. Physiol.*, **76**: 368–378.

KROGH, A., 1937. Osmotic regulation in fresh water fishes by active absorption of chloride ions. *Zeit. vergl. Physiol.*, **24**: 656–666.

LIU, C. K., 1942. Osmotic regulation and "chloride secreting cells' in the paradise fish, *Macropodus opercularis*. *Sinensia*, **13**: 15–20.

LUDFORD, R. J., 1926. Further modifications of the osmic acid methods in cytological technique. *J. Roy. Micro. Soc.*, **46**: 107–109.

PETTENGILL, OLIVE AND D. EUGENE COPELAND, 1948. Alkaline phosphatase activity in the chloride cell of *Fundulus heteroclitus* and its relation to osmotic work. *J. Exp. Zool.*, **108**: 235–242.

SCHLIEPER, CARL, 1933. Über die osmoregulorische Funktion der Alkiemen. *Zeit. vergl. Physiol.*, **18**: 682–695.

SMITH, HOMER W., 1930. The absorption and excretion of water and salts by marine teleosts. *Am. J. Physiol.*, **93**: 480–505.

HETEROPLOIDY IN TRITURUS TOROSUS. II. THE INCIDENCE OF CHROMOSOMAL VARIATIONS IN SHIPPED LARVAE[1]

DONALD P. COSTELLO AND CATHERINE HENLEY

University of North Carolina

Studies on the incidence of heteroploidy and other cytological abnormalities in salamander larvae have been given considerable impetus in recent years by the work of Fankhauser and his students. Such studies have been concerned with the experimental production of chromosomal aberrations, as well as with their spontaneous occurrence in natural populations of amphibian larvae. A general review of the results obtained before 1945 is presented by Fankhauser (1945).

The eggs of the California newt, *Triturus torosus*, are laid in "clutches" which are available in large numbers near Stanford University. Since no systematic effort had been made to determine the spontaneous incidence of heteroploidy in this form, it was decided in 1947 to undertake such a survey. Accordingly, a number of egg masses were sent by Railway Express from Palo Alto to Chapel Hill, in a thermos jug containing ice and water; this is the usual method for shipping amphibian eggs. Cytological examination of the larvae developing from these eggs revealed a high frequency of chromosomal mosaicism and very radical mitotic anomalies.

A comprehensive study was begun early in 1948 in order to eliminate the possibility that such aberrations might be present in material not subjected to shipment. Tail-tips from larvae fixed in California had only a very low incidence of abnormality, suggesting that factors involved in shipment might be responsible for the observed effects. During the period when this control material was being fixed and studied in California, another group of *T. torosus* eggs was shipped east to Chapel Hill. Egg clutches collected at the same time and in the same locale as these "1948 shipped eggs" were raised under known conditions, so that a specific control for this second group of shipped larvae was available. The data from a study of the control animals have been presented in detail by Costello and Henley (1949).

The present paper will describe the results of a study of the original (1947) group of shipped larvae, as well as the second (1948) group.

Preliminary reports concerning small random samples of these shipped larvae have been noted in abstracts (Costello and Henley, 1947a, 1947b, 1948; Costello, 1948).

We are indebted to Dr. Victor C. Twitty and Dr. H. E. Lehman for their kindness in sending us the original batch of shipped eggs in 1947.

---

[1] This work was made possible by grants from the American Philosophical Society, and the Carnegie and Smith Research Funds of the University of North Carolina.

METHODS

1. 1947 material. Unfortunately, no data are available concerning the details of collection and shipment of these eggs. It is probable that they were in quite advanced stages of development at the time of shipment, since most of the embryos were neurulae and tail-buds when received in Chapel Hill approximately one week later. At the time of receipt, all ice in the thermos jug had melted, but the water was still cool.

Upon their arrival in Chapel Hill, the egg clutches were placed in large fingerbowls of spring water and kept in a cold room at approximately 13° C., until the time of the first tail-clipping. The eggs were removed from the cold room and allowed to return to laboratory temperature from 1 to 24 hours prior to the amputation of the tail. After this initial clipping, they were raised at laboratory room temperature, two per dish, in small fingerbowls of spring water.

At least two photographs were made of each larva, to serve as records of their appearance and gross characteristics. MS–222 or chloretone (both made up 1 : 2000 in distilled water) was used to immobilize the larvae for photographing and for the subsequent tail-clipping. The general technique utilized for amputating and staining the tail-tips has been described earlier (Costello and Henley, 1949). In many cases, three successive tips were obtained from each individual and prepared for cytological study; they were clipped at 10 to 14 day intervals. All the animals in this group were fed cladocera and Tubifex. Four hundred fifty-nine "first" tips, 338 "second" tips and 302 "third" tips were studied from these individuals.

2. 1948 material. The 1948 larvae were derived from eggs collected at Los Trancos, California, as late cleavage stages and blastulae. Fifty clutches were shipped from Palo Alto to Chapel Hill the day following collection, in "heavily iced" thermos jugs of lake water (15 ice cubes + 500 cc. lake water). Fifty other clutches collected at the same time and in the same locale were maintained at 12° to 15° C., and were kept as specific controls for the shipped eggs.

When the eggs arrived in Chapel Hill one week after shipment, they were placed in large fingerbowls of spring water. Some of the embryos were raised at laboratory room temperature, and some in the cold room, at about 6° C. After photography and the initial tail-clipping, they were assigned serial numbers and kept individually in small fingerbowls of spring water. The regenerated tail-tip and, in some cases, a "third" tip (second regenerate) were clipped and fixed at approximately 12 to 14 day intervals. Cladocera and Enchytrea were used as food for these larvae. Three hundred seventy-nine "first" tips," 269 "second" tips, and 54 "third" tips from the 1948 shipped animals were studied cytologically.

All tail-tips from both the 1947 and 1948 groups of larvae were examined, using the procedure outlined earlier (Costello and Henley, 1949). Each tip was first studied at a magnification of 210 × ; nuclear size outlines were then drawn with the aid of the camera lucida, using 10 × oculars and an oil immersion objective. Wherever possible, the number of nucleoli in each nucleus drawn was determined in the 1948 tips; the possible value of this criterion had not been foreseen at the time when the 1947 tips were studied. Chromosome counts were established using 15 × compensating eyepieces, an oil immersion objective and the camera lucida (total magnification 2100 ×).

The tips were classified utilizing the criteria discussed by Costello and Henley (1949): 1) Nuclear size and nucleolar number; 2) the presence of abnormal mitotic configurations; and 3) the presence of heteroploid metaphases checked, whenever possible, by chromosome counts. In many cases, more than one indication of abnormality was observed; under such circumstances classification was based on the most radical and clearly demonstrable of the atypical features.

RESULTS

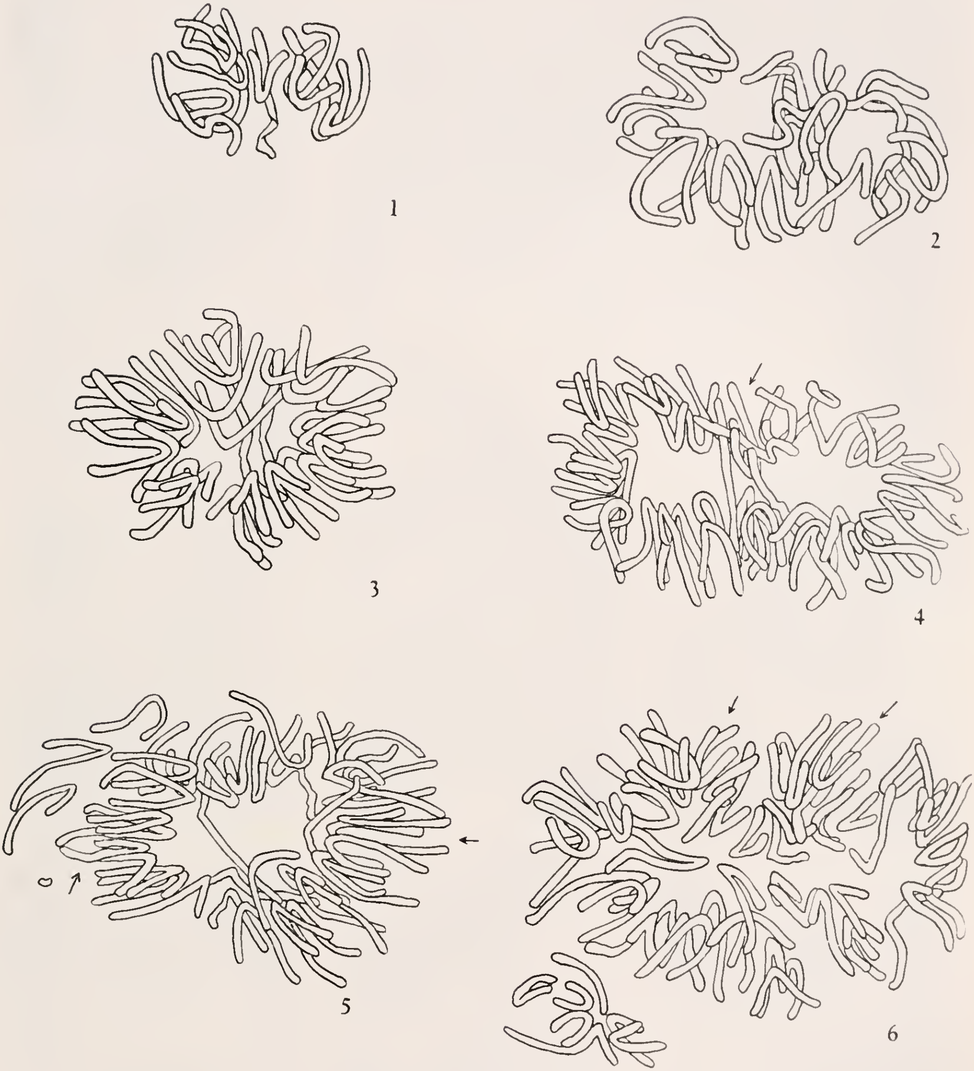
A summary of the data concerning shipped and control larvae is contained in Table I.

TABLE I

The incidence of cytological abnormality in shipped Triturus torosus larvae

Clipping	Total no. tips	Normal diploids		Variable nuclei		Abnormal mitoses		Mosaics	
1947 "First" tips	459	215	46.8%	213	46.4%	21	4.6%	10	2.2%
1948 "First" tips	379	166	43.8%	146	38.5%	54	14.2%	13	3.4%
Total	838	381	45.5%	359	42.8%	75	8.9%	23	2.7%
1948 Control "First" tips	582	423	72.6%	105	18.0%	53	9.1%	1	0.17%
1947 "Second" tips	338	142	42.0%	81	23.9%	39	11.5%	76	22.5%
1948 "Second" tips	269	98	36.4%	107	39.8%	55	20.4%	9	3.3%
Total	607	240	39.5%	188	30.9%	94	15.5%	85	14.0%
1948 Control "Second" tips	429	284	66.2%	88	20.5%	55	12.8%	2	0.5%
1947 "Third" tips	302	138	45.7%	126	41.7%	27	8.9%	11	3.6%
1948 "Third" tips	54	15	27.8%	33	61.1%	5	9.3%	1	1.8%
Total	356	153	43.0%	159	44.6%	32	9.0%	12	3.4%

It is apparent that the incidence of all types of abnormality is roughly comparable among the first and third tips. Furthermore, the frequency of atypical features in first and third tips of both groups of shipped larvae and in the control larvae is not nearly so pronounced as in the second tips. It has previously been pointed out that second tips are far superior to both first and third tips for cytological study (Costello and Henley, 1949), and that among control animals the occurrence of chromosomal aberrations is much more frequent in second tips. The somewhat greater incidence of abnormal mitoses among control first tips (9.1%) than in the first tips of shipped material (8.9%) is surprising. However, this apparent discrepancy can perhaps be explained by the fact that the 1948 control tips were clipped at a slightly later time than the shipped tips. They are characterized in general by a somewhat larger number of mitoses, increasing the chances that abnormalities would be noted. Among the second tips, the occurrence of cytological abnormality



Explanation of Figures

Figures 1–6. Camera lucida drawings of representative metaphase figures in tail-tips of *T. torosus* larvae subjected to shipment. The arrows indicate regions where additional chromosomes were present but too obscure to be traced along their entire length. Figure 1: Haploid; 11 chromosomes counted. Figure 2: Diploid; 22 chromosomes counted. Figure 3: Triploid; 30 chromosomes counted. Figure 4: Tetraploid; 42 chromosomes counted. Figure 5: Pentaploid; 46 chromosomes counted. Figure 6: Octaploid; 65 chromosomes counted. Drawn at a magnification of 2100 ×, reduced by engraver to 1250 ×.

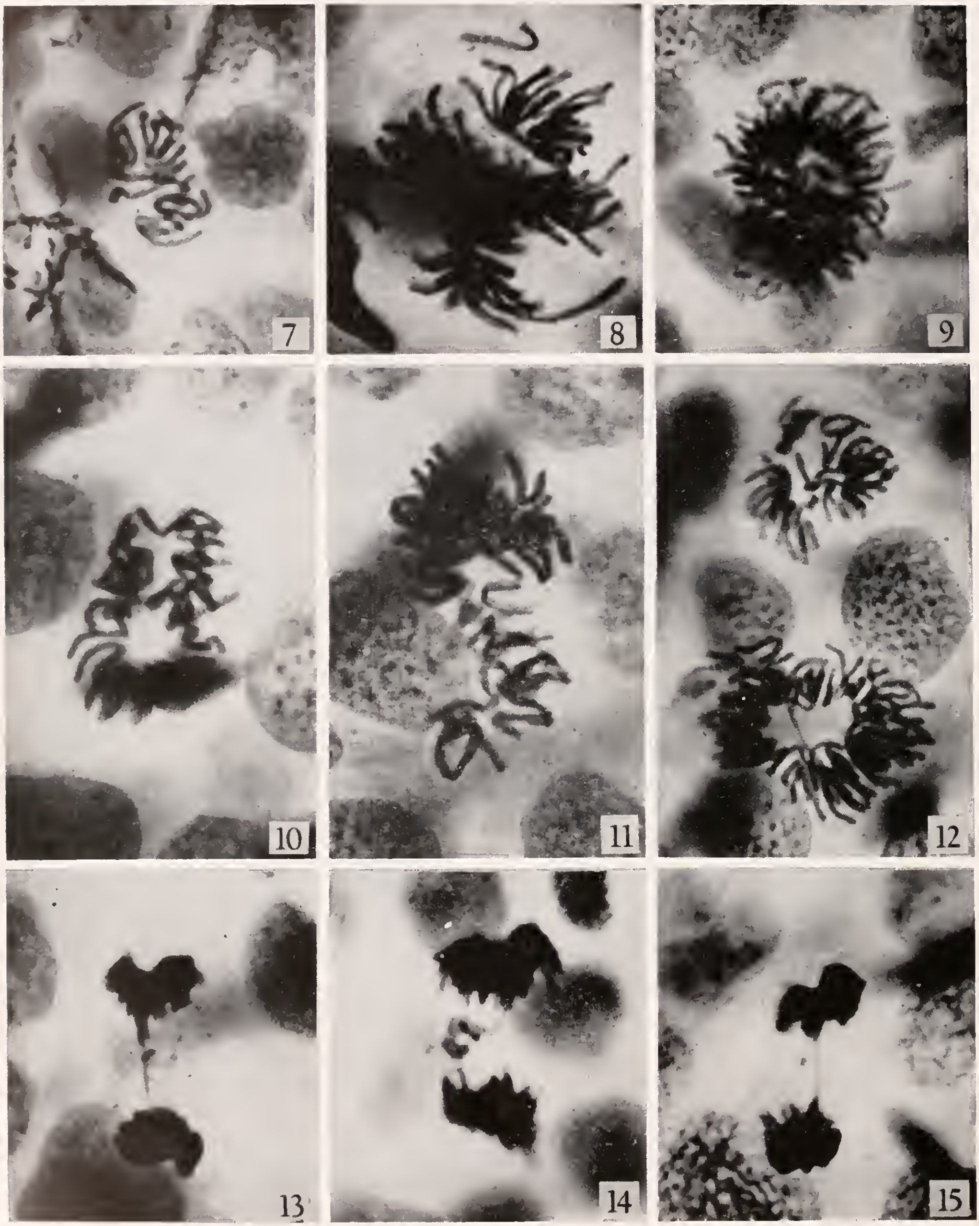


PLATE I

Photomicrographs of mitotic figures from tail-tips of shipped *T. torosus* larvae. Magnifications: Figures 7, 8, 14: 1100 ×; Figures 9, 12: 960 ×; Figures 10, 11, 13, 15: 1160 ×. Reduced 20% off.

FIGURE 7. Haploid metaphase (same as Fig. 1), with 11 chromosomes counted.

FIGURE 8. Pentaploid metaphase, with 46 chromosomes counted. Most of the chromosomes in the figure are split.

in the 1947 and 1948 shipped material is higher than in the control material; the number of mosaics is notably higher.

The most significant data are probably those concerned with the second tips, as indicated in Table I. Among the 338 second tips clipped from the shipped larvae in 1947, 142 (42.0%) were normal diploids, 81 (23.9%) had nuclei of variable size, 39 (11.5%) had abnormal mitoses and 76 (22.5%) were chromosomal mosaics. This is a remarkably high frequency of mosaicism, although it is lower than that reported by us in preliminary notes for much smaller random samples of these same tips (Costello and Henley, 1947a, 1947b, 1948). Of the 269 second tips studied from the 1948 shipped material, 98 (36.4%) were normal diploids, 107 (39.8%) had nuclei of variable size, 55 (20.4%) had abnormal mitoses, and 9 (3.3%) were chromosomal mosaics. The percentage occurrence of aberrations when the 1947 and 1948 groups are taken as a whole is obviously considerably greater than that recorded for second tips from control larvae fixed in 1948. Especially striking is the 14.0% incidence of chromosomal mosaicism, as compared with 0.5% for the control animals.

Mosaicism. A wide variety of types of mosaics was found. Among the 1947 first tips from shipped larvae, there were four haploid/diploids, one diploid/hyperdiploid, four diploid/triploids, and one diploid/tetraploid. Eight of these 10 mosaics were confirmed by exact chromosome counts. In all, 55 of the 76 cases of mosaicism observed among the 1947 shipped second tips were checked by direct chromosome counts. The 76 mosaics included the following: one haploid/diploid; four hypodiploid/diploids; two hypodiploid/diploid/hyperdiploids; one hypodiploid/diploid/tetraploid; 20 diploid/hyperdiploids; 13 diploid/triploids; 19 diploid/tetraploids; three diploid/pentaploids; four diploid/hexaploids; one diploid/octaploid; one diploid/triploid/tetraploid; four diploid/tetraploid/pentaploids; one diploid/tetraploid/hexaploid; one diploid/triploid/tetraploid/pentaploid; one diploid/hyperdiploid/tetraploid. Tail-tips of many of these complex mosaics showed some abnormal mitoses.

Nine chromosomal mosaics were found in the group of second tips from the 1948 shipped larvae, and 5 of these 9 were checked by chromosome counts; among them were one haploid/diploid; one diploid/hyperdiploid; 5 diploid/triploids; one diploid/tetraploid; and one diploid/pentaploid.

There were 11 cases of heteroploid figures in third tips of the 1947 material; two were checked by direct chromosome counts. The remainder were established by estimates of the number of chromosomes present in figures too crowded for exact

---

FIGURE 9. Possible hexaploid metaphase. The figure was too crowded for an accurate chromosome count to be practicable.

FIGURE 10. Hypodiploid metaphase. Twenty chromosomes were counted, and the figure was sufficiently well-spread that this count is probably reliable.

FIGURE 11. Triploid metaphase, with 32 chromosomes counted.

FIGURE 12. Diploid (upper) and pentaploid (lower) (same as Fig. 5) metaphases in adjacent epidermal cells. There were 46 chromosomes counted in the pentaploid figure.

FIGURE 13. "Sticky" anaphase, with deleted fragment and bridge.

FIGURE 14. Anaphase with four lost chromosomes or chromosome fragments. At least two of these deleted elements appear to be complete or nearly complete chromosomes.

FIGURE 15. "Sticky" anaphase with a deleted chromosome fragment attached by a very fine strand of chromatin to the upper group of chromosomes.

counting. Of these 11 mosaics, one was hypodiploid/diploid; four were diploid/triploid; four were diploid/pentaploid; and two were diploid/hexaploid. The single mosaic found in the third tips from the 1948 shipped larvae was hypodiploid/diploid.

All aneuploid mosaic constitutents listed above were established by chromosome counts, since such counts are the only reliable method for ascertaining chromosome number in metaphases where only one or two chromosomes have been added to or lost from the diploid chromosome complement.

The bizarre combinations of chromosome numbers were among the striking features of the complex mosaics. In almost every case, the heteroploid metaphases occurred as isolated figures in single cells, rather than in patches of heteroploid tissue. Similarly, the sporadic occurrence of very large interphase nuclei among nuclei of normal size is evidence that, in general, the mosaic cells are not present as sizable areas of tissue. Larvae possessing these features can be characterized as predominantly diploid, but with some heteroploid cells making them mosaics.

Nuclear abnormalities. Many of the interphase nuclei showed evidence of earlier mitotic abnormality by the presence of blebs of extruded chromatin, "amoeboid" lobes, or slender connecting bridges between two adjacent nuclei. Quite frequently the periphery of the nucleus stained intensely with haematoxylin, while the central region was more nearly homogenous. Comparable configurations have been reported by us as occurring in control larvae collected from very cold water (see Fig. 17 in the paper by Costello and Henley, 1949), and in experimentally cold-treated larvae (Henley, 1950).

Mitotic abnormalities. A remarkable number of multipolar spindles was observed in tail-tips from the larvae subjected to shipment; examples are shown in

---

PLATE II

Photomicrographs of epithelial nuclei and mitoses from tail-tips of shipped *T. torosus* larvae. Magnifications: Figure 16: 1100 ×; Figure 17: 560 ×; Figure 23: 960 ×; all other figures: 1160 ×. Reduced 20% off.

FIGURE 16. Nuclear débris resulting from the breakdown of epithelial nuclei. The dark globules are intensely basophilic. Small areas of such débris ("necrotic areas") are occasionally found in tail-tips from control larvae, but are not usually so extensive nor so frequent in occurrence as in shipped and experimentally cold-treated animals.

FIGURE 17. Cell boundaries in the tail-tip epithelium. This preparation was made utilizing a silver impregnation method, and the nuclei are not stained. The characteristic shape of the cells is clearly evident, as are quite pronounced variations in cell size.

FIGURE 18. Tripolar configuration of chromosomes. Superficially, this figure appears to be in anaphase, but the morphology of the chromosomes appears to be more typical of metaphase. A total of 18 chromosomes was counted. The outline of the cell boundary is quite clearly defined.

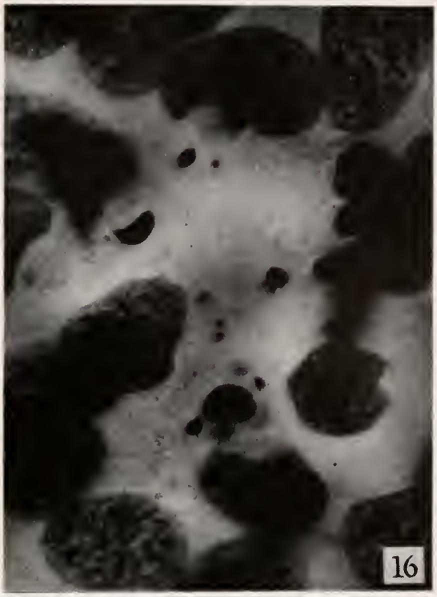
FIGURE 19. Tripolar telophase. A fine connection is present between the two upper groups of chromosomes.

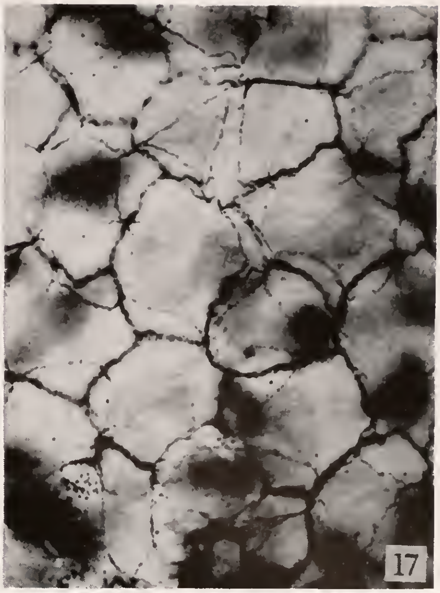
FIGURE 20. Tetrapolar figure, displaced around a highly vacuolated cell. The necrotic nucleus of this vacuolated cell is visible in the center of the clear area.

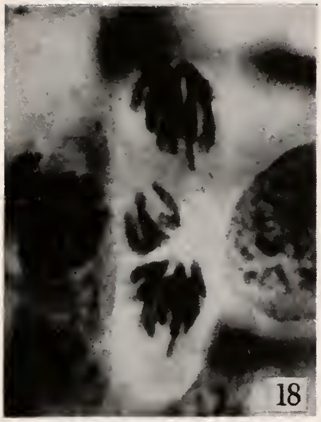
FIGURE 21. Tripolar anaphase, with several persistent bridges between the two lower groups of chromosomes. The dark granules at the bottom of the photomicrograph are nuclear débris.

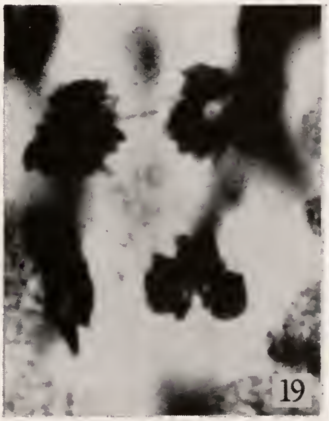
FIGURE 22. Tripolar anaphase, similar to that shown in Figure 18, but representing a slightly later stage. Nineteen chromosomes were counted.

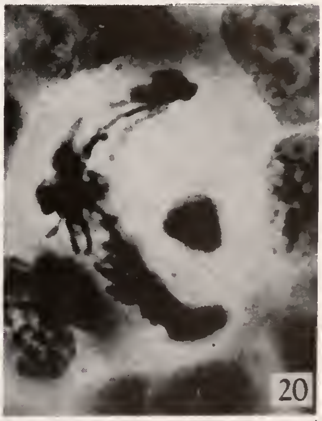
FIGURE 23. Tetrapolar anaphase, in which a total of 75 chromosomes was counted. One ring chromosome is visible at the right.

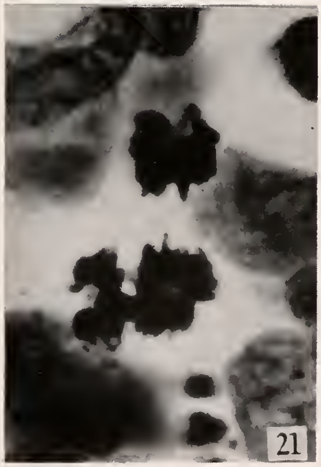

16

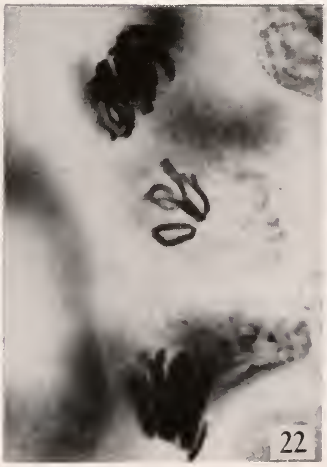

17


18


19


20


21


22

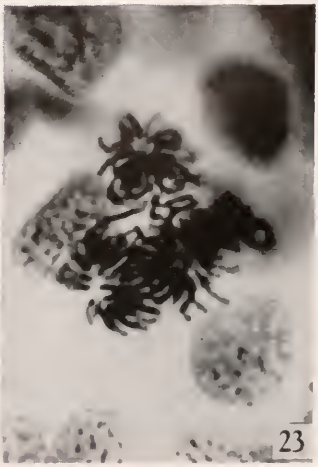

23

Plate II

Figures 18–21 and Figure 23. Seven clear tripolar anaphases and telophases, and 10 tetrapolar spindles were found on first, second and third tail-tips of the 1947 and the 1948 shipped material. No such configurations have been found in tail-tips from control larvae of this species.

Another commonly observed type of aberration was the presence of relatively uncondensed masses of chromatin. Similar configurations have been reported in experimentally cold-treated *T. torosus* larvae (Henley, 1950), and are also occasionally found in control larvae, especially those collected from very cold water (Costello and Henley, 1949). These chromatin masses resemble abortive prophases. They were sometimes noted near metaphases and appeared to represent chromatin material normally present as chromosomes in a diploid figure.

A number of types of abnormality were observed in metaphases. These included "ragged" figures, in which the chromosomes were slender and thread-like rather than compact and cylindrical; the arrangement of the chromosomes on the spindle in these cases was often irregular. The chromosomes in some metaphases were intensely basophilic and arranged in dense tangled masses very unlike the normal type. Almost invariably these "wreath" configurations were too obscure for chromosome counts to be practicable. A third abnormality observed in metaphase figures was the reversal of one or more chromosomes on the spindle, so that the kinetochore region was directed outward from the center of the ring-shaped figure, rather than toward the center as is normally the case. This condition may foreshadow another frequently observed abnormality, in which the chromosomes had become completely lost from the metaphase spindle and were apparently loose in the cytoplasm. Such "lost" chromosomes or chromosome fragments have been observed in a few of the control preparations (Costello and Henley, 1949), but are not nearly so common there as in these shipped larvae.

Many types of anomalies were observed in anaphase and telophase mitoses, including the multipolar spindles referred to above. Sometimes the two poles of bipolar figures were quite markedly asymmetric, so that one had discernibly more chromatin material than the other. There were also bent spindles, in which the center of orientation of one of the two poles was almost at right angles to that of the other. "Sticky" anaphases and telophases were frequently encountered (Figs. 13, 15), as well as chromosomes and chromosome fragments which appeared to have lost their spindle connections, so that they were left behind in the migration of the daughter elements toward the poles. An extreme example of this type is shown in Figure 14. The loss or disorientation of chromosomes at the poles of anaphase or telophase figures was quite common. In a few instances, anaphases were observed in which large unorganized masses of chromatin material were present on the spindle.

Structural abnormalities of the larvae. The process of shipment appears in some way to have affected the formation of the lateral line organs in these larvae; many cases were found in which displacement of the lateral line condensations of the tail-tip had occurred, similar to those reported for experimentally cold-treated *T. torosus* larvae (Henley, 1950). Often supernumerary condensations were present toward the edges of the tail-fins; these appeared to be morphologically identical with the normal lateral line organs. The significance of these developmental anomalies is thus far obscure.

There was no observable correlation between the general morphology of the larvae and the presence or absence of cytological abnormality. A careful check of the photographs of the larvae indicated no recognizable basis for identifying larvae with either abnormal mitoses or the described types of mosaic cells. This confirms observations reported by us for control *T. torosus* larvae, as well as for experimentally cold-treated animals.

DISCUSSION

I. Possible factors involved in the production of abnormalities

The foregoing results indicate clearly that a higher percentage of various nuclear and mitotic abnormalities is found in shipped than in control material. The major question for discussion is concerned with the reasons for such an effect. Among the possible factors contributing to the production of the abnormalities observed in the shipped material, the following may be considered:

a) *Cold.* The eggs were shipped with ice in the thermos jugs, and the temperature of the water presumably remained quite low for an extended period of time. Experimental cold-treatment has been demonstrated to produce similar anomalies in larvae of *Triturus torosus* (Henley, 1950), as well as in other species of salamanders (Böök, 1943, 1945; Fischberg, 1947; *et al.*).

b) *Partial anaerobiosis.* Utilization of most of the available oxygen within the closed jugs could have occurred during the one-week period of transit. This deficiency of oxygen might be especially pronounced near the centers of the jelly masses. There is some evidence to indicate that anoxia is of considerable importance in producing cytological abnormality in tail-tips of *Amblystoma punctatum* larvae (unpublished data).

c) *Accumulation of carbon dioxide.* The gaseous products of metabolism of the embryos do not escape from the closed jug system, from the time of shipment until the time of receipt. Such accumulation of carbon dioxide may have deleterious effects on the embryos, either directly or indirectly, through lowering of the pH of the medium.

d) *Mechanical agitation.* Shipment by Railway Express probably involves almost continuous shaking of the jug contents.

e) *Toxic water.* The ice used was made by freezing ordinary tap water; even though the liquid medium was non-chlorinated lake water (also used for raising the control animals), the water from the melting ice may have introduced toxic factors. In general, it is the experience of the Stanford group of embryologists that larvae which are raised in heavily chlorinated or other unsuitable water are characterized by laterally-curled tail-tips, with deviated tail-fins. No such curled tails were noted in our material, so perhaps this factor may be eliminated from further consideration.

f) *Irradiation.* This remote possiblity is mentioned only because of the similarity between the observed aberrations and the types of irradiation-produced anomalies described by Alberti and Politzer (1924a, 1924b). We have no evidence that any such radiations were present, and it would be a remarkable coincidence if they were operative, even in different dosages, during two separate salamander breeding seasons.

g) Anaesthesia and other factors inherent in the process of photographing the larvae. Theoretically, the anomalies found might be attributed to the effects of the anaesthetic required to immobilize the larvae for photography, or to the heat given off by the Photoflood lamps used as a light source. Such effects, if present, would be expected to bear a definite relation to the time of subsequent clipping. However, they can probably be ruled out, on the basis of cytological studies of tail-tips from *Amblystoma punctatum* larvae which were not subjected to anaesthesia or photography (unpublished data).

II. The correlation between cytological abnormality present on first, second and third tail-tips

On the assumption that the factors responsible for mitotic anomalies were present during the period of egg shipment, as indicated above, the data were examined to ascertain the degree of correlation between the occurrence of abnormalities in first, second and third tail-tips. If the factors producing abnormality were operative during the period of late cleavage, gastrulation and neurulation, we should expect to find some regularity in the subsequent events taking place in the epidermal cells of the growing larvae. There are several possibilities: 1) All three tail-tips of an affected larva might have a similar high percentage of abnormal figures. 2) The three tail-tips could exhibit a progressive increase in the number of abnormal figures, due to an increase in the number of cell progeny of the affected cells. 3) There might be a progressive decrease in the number of atypical mitoses in the three tips. This would obtain if the abnormal cells ceased to divide further and, becoming necrotic, were eliminated from the tip.

The data indicate that very little correlation exists. Only three second tail-tips were mosaic among the 23 larvae whose first tips were characterized by that condition. From this same group of 23, not even one third tail-tip was found to have heteroploid metaphases. Of the 85 larvae with mosaic second tips, three belonged to the group of 23 with mosaic first tips. A single animal of the group of 85 showed mosaicism in its third tail-tip. Only one individual of the 12 which had mosaic third tips was demonstrated to have had heteroploid figures present before the time of the third clipping.

In addition to this lack of correlation, there was no progressive increase or decrease in abnormality. Instead, the incidence of atypical figures was found to be much higher in second tips (85 mosaics) than in "firsts" (23) or "thirds" (12). How can this be explained?

The second tips are by far the most favorable material, for several reasons. They are relatively free of yolk, whereas the first tips may have some yolk-obscured cells, especially if clipped a little too early. Furthermore, the incidence of mitosis is very much higher in the second tips than in either "firsts" or "thirds." This may be due to the onset of a period of rapid growth and cell multiplication concurrent with the beginning of feeding; such a period presumably tapers off after about 10 days. Another possibility is that there is more rapid cell division following the stimulation of the initial tail-clipping, which persists for as much as 12 to 14 days. However, if this be true, it seems strange that a similar stimulation of mitosis does not follow the second amputation. Finally, one must consider the fact that the second tips have noticeably fewer pigment cells than the "firsts" (but not fewer than the "thirds"), so that a smaller number of figures would be obscured. This finding, in itself, obviously does not account for the differences between the "seconds" on the one hand, and the "firsts" and "thirds" on the other.

The frequent occurrence in this material of necrotic areas, with nuclear débris resulting from partial or complete disintegration of epithelial nuclei, is an established fact. A typical example of this phenomenon is figured (Fig. 16). The staining characteristics of such areas are described briefly by Costello and Henley (1949). We have no way of knowing whether or not nuclear necrosis is initiated in abnormal cells. It appears that arrested nuclear disintegration could be an effective cause of hypodiploid nuclei.

It must be concluded that, at the present time, we cannot adequately account for the high percentage of deviations from normal mitotic conditions. Neither can we account for the higher incidence of abnormalities found in the 1947 shipped material, as compared with the larvae subjected to shipment in 1948. We do know, however, that the 1948 embryos were kept under more carefully controlled conditions before shipment. If the radical aberrations described herein are the result of such minor environmental changes as obtain in the ordinary procedures for handling and shipping embryological materials, it is of vital importance to emphasize this fact. Many amphibian embryologists working in this country today are experimenting with cytologically sensitive and perhaps abnormal material.

SUMMARY

1. Tail-tips from *Triturus torosus* larvae subjected to shipment by Railway Express have been studied cytologically.

2. A total of 838 "first" tips was examined. Of these, 381 (45.5%) were normal diploids, 359 (42.8%) had nuclei of variable size and/or variations in nucleolar number, 75 (8.9%) had abnormal mitoses, and 23 (2.7%) were chromosomal mosaics.

3. Of the 607 regenerated "second" tips studied, 240 (39.5%) were normal diploids, 188 (30.9%) had nuclei of variable size and/or variations in nucleolar number, 94 (15.5%) had abnormal mitoses, and 85 (14.0%) were chromosomal mosaics.

4. Three hundred fifty-six second regenerates ("third" tips) were examined. Among these, 153 (43.0%) were normal diploids, 159 (44.6%) had nuclei of variable size and/or variations in nucleolar number, 32 (9.0%) had abnormal mitoses, and 12 (3.4%) were chromosomal mosaics.

5. The following types of mosaics were found: haploid/diploid; hypodiploid/diploid; hypodiploid/diploid/hyperdiploid; hypodiploid/diploid/tetraploid; diploid/hyperdiploid; diploid/triploid; diploid/tetraploid; diploid/pentaploid; diploid/hexaploid; diploid/octaploid; diploid/triploid/tetraploid; diploid/tetraploid/pentaploid; diploid/tetraploid/hexaploid; diploid/triploid/tetraploid/pentaploid; diploid/hyperdiploid/tetraploid. These mosaics were predominantly diploid, with, for the most part, isolated heteroploid cells in scattered areas.

6. Seventeen instances of multipolar anaphases and telophases were found in the tail-tips from these shipped larvae. Seven of these were tripolar and 10 were tetrapolar.

LITERATURE CITED

Alberti, W. and G. Politzer, 1924a. Über den Einfluss der Röntgenstrahlen auf die Zellteilung. *Arch. Mikroskop. Anat. u. Entw.*, **100**: 83–109.

Alberti, W. and G. Politzer, 1924b. Über den Einfluss der Röntgenstrahlen auf die Zellteilung. II. Mitteilung. *Arch. Mikroskop. Anat. u. Entw.*, **103**: 284–307.

Böök, J. A., 1943. Low temperature and cleavage in *Triton taeniatus*. *Hereditas*, **29**: 195–197.

Böök, J. A., 1945. Cytological studies in Triton. *Hereditas*, **31**: 177–220.

Costello, D. P., 1948. Report of Progress. *Year Book Amer. Phil. Soc. for 1948:* 122–124.

Costello, D. P. and C. Henley, 1947a. Abnormalities of mitosis in tail-tips of *Triturus torosus*. *Biol. Bull.*, **93**: 205–206.

Costello, D. P. and C. Henley, 1947b. Mitotic abnormalities in tail-tips of *Triturus torosus*. *Anat. Rec.*, (Suppl.) **99**: 26–27.

Costello, D. P. and C. Henley, 1948. A comparison of heteroploidy in natural and shipped *Triturus torosus* embryos. *Anat. Rec.*, **101**: 52–53.

Costello, D. P. and C. Henley, 1949. Heteroploidy in *Triturus torosus*. I. The incidence of spontaneous variations in a "natural population." *Proc. Amer. Phil. Soc.*, **93**: 428–438.

Fankhauser, G., 1945. The effects of changes in chromosome number on amphibian development. *Quart. Rev. Biol.*, **20**: 20–78.

Fischberg, M., 1947. Experimentelle Auslösung von Heteroploidie durch Kältebehandlung der Eier von *Triton alpestris* aus verschiedenen Populationen. *Genetica*, **24**: 213-329.

Henley, C., 1950. Cytological abnormalities produced by experimental temperature shock on *Triturus torosus* embryos. *Biol. Bull.*, **98**: 1–18.

SPERM ENTRANCE IN ECHINODERMS, OBSERVED WITH THE PHASE CONTRAST MICROSCOPE[1]

JEAN C. DAN

Biology Department, Tokyo Metropolitan University and Misaki Marine Biological Station[2]

In most animal species it is an accepted fact that the entire spermatozoan, including the tail, enters the egg to accomplish fertilization and, in many cases, has been demonstrated within the egg cytoplasm of stained sections (Fig. 1) (Selenka, 1878; Meves, 1915; Yatsu, 1909; Kostanecki and Wierzejsky, 1896). In echino-

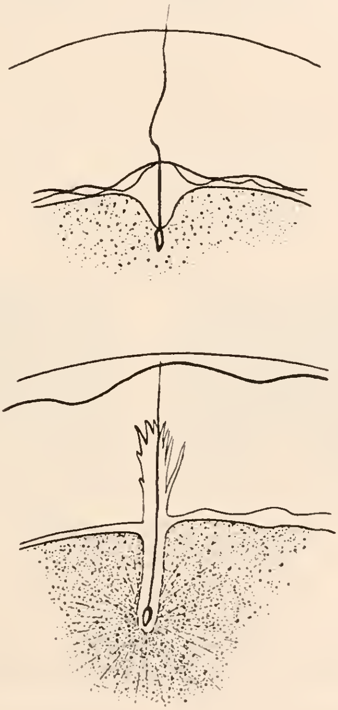


FIGURE 1. Sperm penetration into the egg of *Toxopneustes variegatus,* as drawn from living material by E. Selenka in 1878.

---

[1] The phase contrast microscope used in this and the following study was purchased with the aid of a grant from the American Philosophical Society in 1947. The writer wishes to express sincerest thanks to the Society, in particular to Prof. E. G. Conklin, and to the Bausch and Lomb Optical Company, for their exceedingly kind cooperation in making the instrument available ahead of their production schedule, at the expense of considerable inconvenience and extra effort on the part of their staff.

[2] The writer also wishes to thank the Director and Staff of the Misaki Marine Biological Station for the use of Station facilities.

derms, however, Wilson (1895) found the sperm tail being carried away on the outside of the rising fertilization membrane (Toxopneustes) and he was unable to detect it within the cytoplasm of either living or fixed eggs. Consequently, although he cites the work of Ries (1909) to the contrary in his book (which since 1925 has served as the definitive treatise of cytology), his lack of certainty in the matter has created a general impression that echinoderms are an exception to the rule that the entire spermatozoan enters the egg.

The later work of R. Chambers (1933) with *Echinarachnius parma* confirms Wilson's observations. Chambers found that the tail of the partially engulfed spermatozoan is gripped by the expanding fertilization membrane and either carried away in entirety from the egg surface as in Wilson's figure, or held until the advance of the head portion has created sufficient tension to cause the tail to break at some point within the membrane. Chambers sidesteps the final decision as to whether any part of the sperm tail enters the egg cytoplasm, by stating that the truncated portion of the tail was "lost among the processes of the exudation cone" (p. 132). However, Chambers presents one case, involving an "unusually active" spermatozoan, in which ". . . the entire length of the sperm tail came to lie within the membrane," and eventually was visible moving within the egg cytoplasm (Fig. 2).

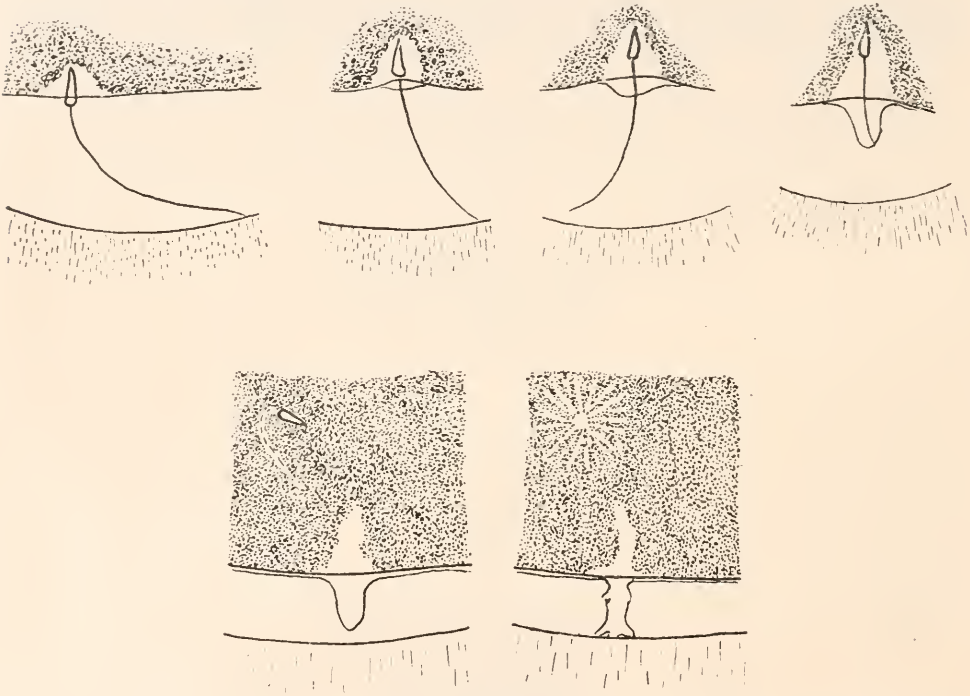


FIGURE 2. Sperm penetration in *Echinarachnius parma*, after R. Chambers, 1933. Chambers describes this as "a case in which the sperm was unusually active" (p. 133).

In view of the important status held by echinoderm eggs as a result of the volume of research which has centered about them, there seemed sufficient reason for bringing improved microscopical technique to bear on the settlement of this old discrepancy in observation.

MATERIAL AND METHODS

Since the phase contrast microscope permits highly accurate observation of such objects as living spermatozoa, it was used in a comparative study of spermatozoan entry in the echinoderm species readily available at the Misaki Marine Biological Station. These include the sea urchins, *Strongylocentrotus pulcherrimus, Heliocidaris crassispina, Clypeaster japonica, Mespilia globulus* and *Pseudocentrotus depressus*, and the starfish, *Asterina pectinifera.*

In order to observe the entire process of insemination, the following procedure was adopted. A small drop of sea water containing the eggs to be fertilized was placed on a glass slide on the microscope stage. A cover glass was coated with saliva because it was found that this prevented the trapping of the spermatozoa against the glass surface—a phenomenon which, if not checked, simultaneously interfered with visibility and reduced the number of sperm available for fertilization.

A very small amount of a dilute fresh sperm suspension was spread on the coated cover glass, and this was placed over the egg-sea water drop. With practice it was found possible to control the amount of sea water so that there was very little movement of the eggs by the time the focus was adjusted. Using a medium low power objective, a field containing several eggs was surveyed to catch a sperm about to enter an egg exactly on the periphery of the largest optical section; the process was then followed with the high dry (43 ×) or the oil immersion (97 ×) objective. Best observation of the sperm tail and fertilization cone was achieved with the high dry objective. It was ordinarily possible to begin observations within about 20 seconds, just as the spermatozoa were penetrating the jelly and approaching the egg surfaces.

RESULTS

In the six species of echinoderms used in this study, it was found that without exception the entire spermatozoan, including the tail, entered the egg in a continuous process, usually requiring from 2 to 4 minutes.

For the sake of continuity, although this necessitates repetition of facts already well known to students of the fertilization process, a composite account will be presented of the phenomena observed in any or all of the species under study.

Penetration of the vitelline membrane

As the spermatozoa begin to arrive at the surface of the unfertilized egg, pushing through the jelly with a slightly rotational swimming movement, one will be seen in which the head, almost immediately after reaching the egg, assumes a position perpendicular to the surface. Although the tail may persist in its vigorous motion, there is no communication of this to the head, which seems to have established a rigid connection with the egg protoplasm, presumably having already made an opening in the metaplasmic vitelline membrane.

In spite of the fact that *Asterina* is a starfish and its eggs are in the germinal vesicle stage when insemination takes place, there was no significant difference observed in the manner of sperm entry between it and the five sea urchin species.

Wave of cortical granule breakdown

The reaction of the egg to the presence of the spermatozoan which has thus made contact with its living protoplasm begins at once, with a visible wave which travels around the egg at speeds varying with the species, but in those measured (*Mespilia, Pseudocentrotus*) roughly of the order of 10 μ sec. This is undoubtedly the "wave of cortical granule breakdown" of Moser, 1939; Motomura, 1941; Runnström, 1948.

In the relatively fluid eggs of Mespilia, the passage of this wave is especially striking; it causes a slight deformation of the surface layers of the egg, which gives the impression that some sort of tension is being progressively released, or that a local band of contraction and expansion is passing around the egg. The effects of this phenomenon are not confined to the outermost layers; eggs which have been stratified and elongated by centrifugation lose much of their stratification and regain a spherical contour as a result of the passage of this wave.

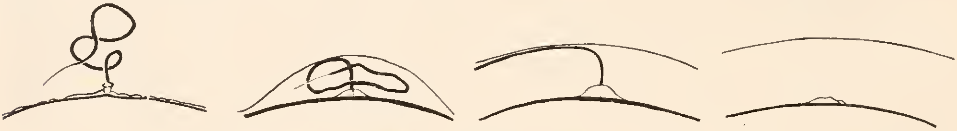


FIGURE 3. *Pseudocentrotus depressus.* The first stage of sperm entrance is characterized by vigorous writhing activity which draws the entire length of the tail within the fertilization membrane as soon as a small perivitelline space has been formed. During the second stage, the tail is relatively quiescent as it is gradually engulfed in the egg cytoplasm.

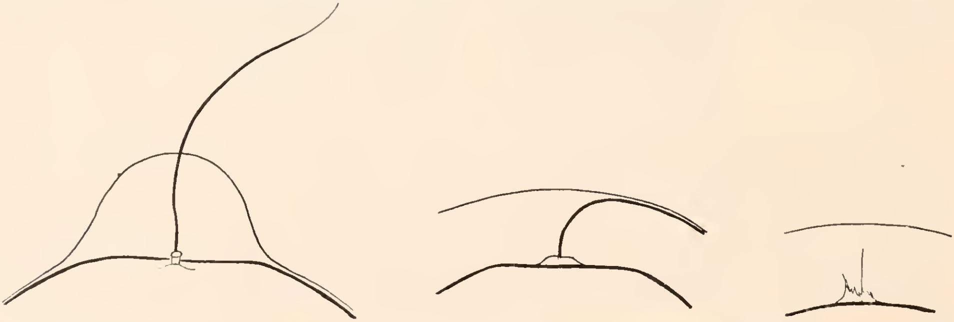


FIGURE 4. *Clypeaster japonica.* In this form the fertilization membrane is lifted rapidly, but the relatively inactive sperm tail is not invariably pulled into the perivitelline space. The originally rounded fertilization cone develops a ragged appearance in the course of degeneration.

Elevation of the fertilization membrane

In four of the species—*Mespilia, Clypeaster, Pseudocentrotus* and *Strongylocentrotus*—a rapid separation of the fertilization membrane is immediately initiated around the point of sperm entry, while the wave of cortical granule breakdown is proceeding around the remainder of the egg. Over an area represented by a little less than one fourth of the egg circumference in optical section, the egg surface is flattened (by the back-thrust of a swelling colloid which pushes up the fertilization membrane?) ; in the center of this area the sperm head is being steadily drawn into the egg cortex.

At the same time, the fertilization membrane, which has been "loosened" around the egg by the first wave, is gradually being pushed away from the protoplasmic surface.

In *Heliocidaris* and *Asterina*, the fertilization membrane is slower in separating, and the perivitelline space does not attain as great a width as that of the other four species.

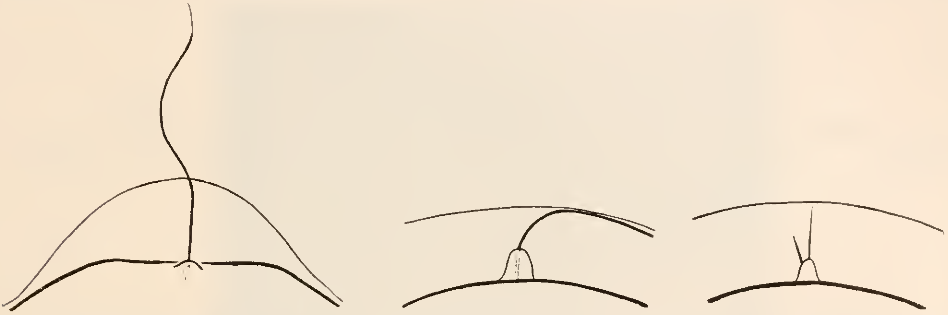


FIGURE 5. *Mespilia globulus.* The fertilization membrane lifts rapidly when the egg is in optimal condition, but the sperm tail may extend through the membrane while it is being drawn into the egg cytoplasm. The degenerating fertilization cone usually has several fine processes.

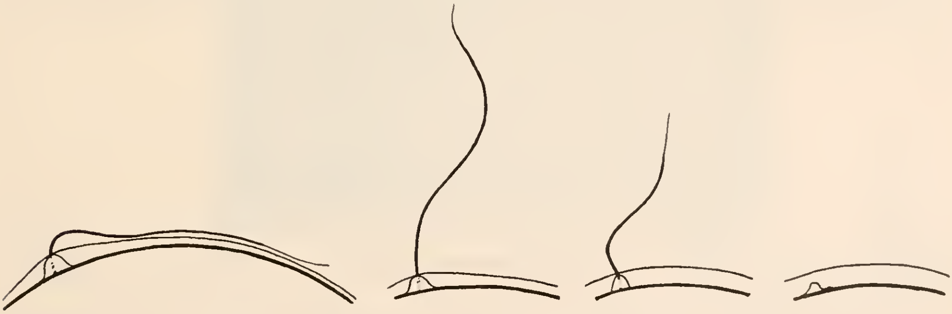


FIGURE 6. *Heliocidaris crassispina.* After the sperm head has penetrated the cortex, the tail lies motionless on the outside of the fertilization membrane for about two minutes; it then straightens out perpendicular to the egg surface and is rapidly drawn into the cytoplasm.

Fertilization cone

The fertilization cones formed in all six species are remarkably similar during what is presumably their functional period (see Figs. 3, 4, 5, 6, 7, 8, 9) ; *i.e.,* while the sperm tail is passing through them into the egg cortex. In each case the cone develops as a mound of hyaline substance after the sperm head has been engulfed into the cytoplasm, and degenerates more or less promptly after the tail has been engulfed.

The shapes taken by the degenerating cones differ rather widely among the various species, from the irregular knobs of *Heliocidaris* (Fig. 6) to the spikes and processes produced by *Clypeaster* (Fig. 4) and *Mespilia* (Fig. 5). *Heliocidaris* and *Asterina* (Fig. 7) are alike in that the fertilization cone develops rapidly to full size and pushes away the slowly separating fertilization membrane at the point of sperm entrance; in the other species full development of the rounded stage is attained somewhat later. The fertilization cones of *Strongylocentrotus* and *Pseudocentrotus* (Fig. 3) subside, or are resorbed into the surface layers of the egg without any spectacular developments in normal cases, although elaborate processes resembling bundles of hyaline fibers were produced in *Pseudocentrotus* eggs treated with nicotine (Fig. 8), and in untreated, immature eggs. Heating to 35° C. also caused the formation of abnormally large cones in *Clypeaster* eggs.

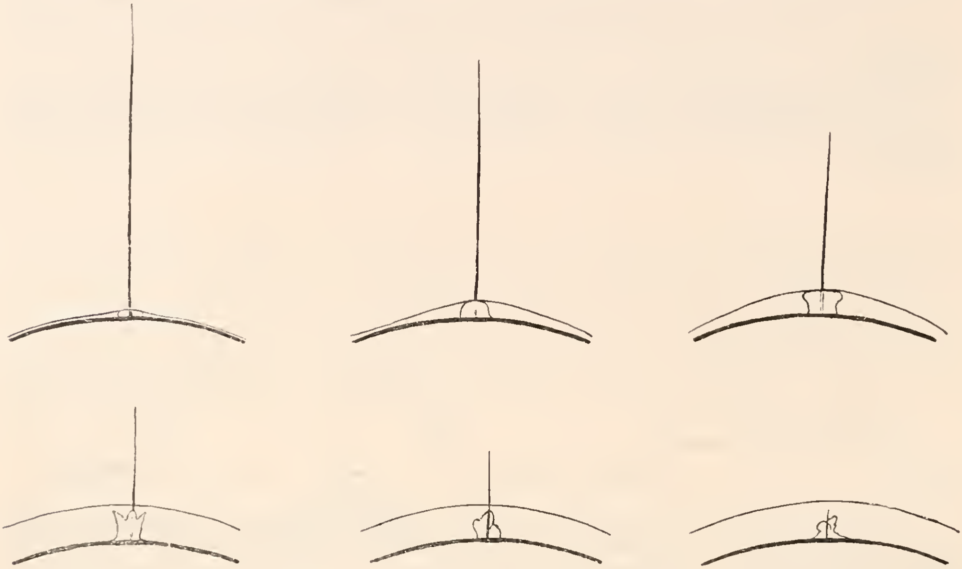


FIGURE 7. Sperm entrance in the starfish, *Asterina pectinifera*. The fertilization membrane lifts off slowly, being pushed up at the sperm entrance point by the fertilization cone. The sperm tail is nearly motionless while it enters the egg.

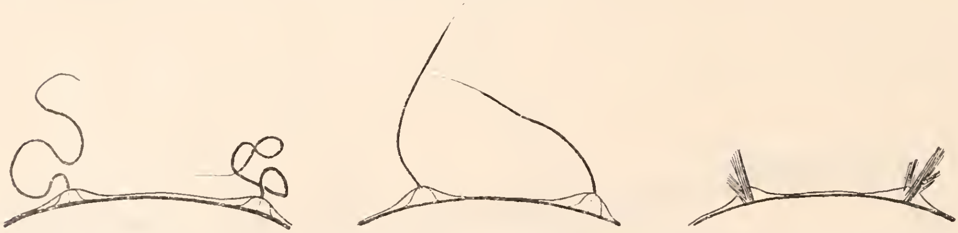


FIGURE 8. Polyspermic fertilization in *Pseudocentrotus* egg treated with nicotine, in which the fertilization membrane is not lifted from the egg surface and abnormally large and persistent fertilization cones are formed. No change from the normal pattern can be detected in the activity of the spermatozoan.

Sperm tail entrance

The degree of activity exhibited by the tails while the spermatozoa are entering the egg cytoplasm varies widely among the species studied. As the consequence of a vigorous writhing motion, the tail of the *Pseudocentrotus* sperm is invariably pulled into the perivitelline space as soon as it is formed. In contrast to this behavior, the sperm tail activity of *Clypeaster, Mespilia* and *Strongylocentrotus* ranges from negligible to spasmodically vigorous, and the tails are often found extending through the fertilization membrane until the length of tail remaining outside the egg becomes less than the width of the perivitelline space. In no species was there observed any phenomenon which could be interpreted as indicating that the fertilization membrane exerted any restraining effect on the sperm tail at any time. From the observations made on these six species it must be assumed that the aperture made in the outermost covering of the egg at the moment of sperm head penetration remains as such, so that the much more slender tail of the sperm is entirely free to slip through it.

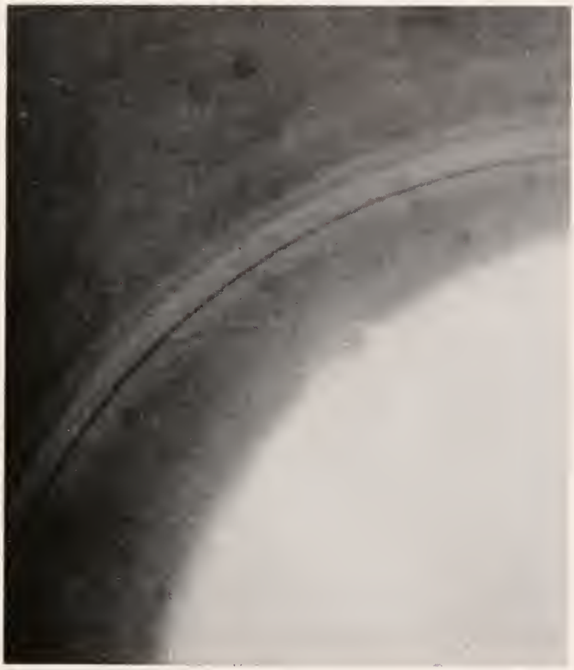


FIGURE 9. Photograph of *Strongylocentrotus pulcherrimus* egg, showing fertilization cone and last stage of sperm tail entrance. Phase contrast microscope, oil immersion (97 ×).

In *Heliocidaris* and *Asterina*, since the perivitelline space is slow to develop and never attains as great a width as that of the other four species, the sperm tail invariably remains outside the fertilization membrane until it is finally engulfed in the fertilization cone (Figs. 6, 7) after about 3½ minutes.

The sperm tail entrance pattern of *Heliocidaris* shows an interesting variation peculiar to its species. After the head has been engulfed, the fertilization membrane lifted off and the fertilization cone formed, the sperm tail, instead of gradually being drawn into the egg, remains lying motionless against the outside of the fertilization membrane (Fig. 6) until about 2½ minutes after fertilization; it then suddently straightens out to a position perpendicular to the egg surface and, moving vigorously, almost the entire length of the sperm tail enters the egg within 15 to 30 seconds. This is the fastest rate observed in any of the species in which the entrance time was recorded.

The time records for the other species, in both of which the tail slowly enters the egg at an approximately uniform rate, are also shown in Table I. These data seem to indicate a faster rate of entrance with rise in temperature within the normal range. However, the data are insufficient to permit the drawing of any conclusion, in view of the amount of variation observed among eggs of the same lots.

TABLE I

Time required for complete engulfment of sperm tail

Species	No. cases	Temp. °C.	Minimum	Maximum	Average
Clypeaster	40	22°–24°	125 sec.	355 sec.	196 sec.
	45	26°–28°	120 sec.	265 sec.	183 sec.
Mespilia	39	27.5°–30°	170 sec.	660 sec.	———
Heliocidaris	18	21.4°–23°	90 sec.	360 sec.	161 sec.
	13	28°	100 sec.	160 sec.	122 sec.

It will be noticed that no average time is given for *Mespilia*. In this form, in about half of the cases observed, the sperm tail was engulfed at a steady rate within 3½ minutes. In the remaining cases, the engulfment of the tail required from 4 to 11 minutes, and, in a few instances, as in Chambers' experience, the tip of the tail "was lost among the processes of the entrance cone," so that it was impossible to fix an accurate time of final engulfment.

Regardless of this desultory behavior on the part of its tail, the sperm head showed no corresponding lag in the formation of the sperm aster and union with the egg pronucleus.

An attempt was made to cause an increase or decrease in the time required for engulfment of the sperm tail in *Heliocidaris, Clypeaster* and *Mespilia* by subjecting the eggs to various abnormal conditions such as chilling (8° C.), warming (35° C.), aging and exposure to polyspermy-producing agents. In no case was a significant change in the entrance time observable. In cases of polyspermy in which two or three sperm entered the egg simultaneously, the entrance time for all the spermatozoa was approximately equal and within the normal range.

During the period covered by the sperm tail entrance into the egg, there are two phenomena which attract the attention: one is the appearance of crystal-like objects in the perivitelline space of *Pseudocentrotus* and *Strongylocentrotus* eggs and the other is a marked "surging" of the entire *Clypeaster* egg mass. The first phenomenon has been intensively studied by Y. Endo, whose report on the subject will be published in the near future.

"Surging" of the cytoplasm—Clypeaster

This mass movement of the cytoplasm has no counterpart, so far as could be determined, among the other species studied, and can hardly be associated with the wave of cortical granule breakdown, since it is initiated after the head of the sperm has completely penetrated the egg cortex—*i.e.,* about 40 seconds after the time of cortical granule breakdown. Starting at this time, the whole mass of egg protoplasm is carried toward the part of the surface where the sperm is being engulfed, so that the contour bulges out in this region. After 30 to 40 seconds this movement slows to a stop, and then immediately begins in the reverse direction, continuing for approximately the same period, again slowing to a stop, reversing its direction and returning to the original equilibrium position in another 30 to 40 seconds. The egg mass has thus completed one period of something approximating simple harmonic motion; and at its conclusion the sperm tail is usually just disappearing into the egg.

This phenomenon is not observed to occur equally in all *Clypeaster* eggs, even under normal (experimental) conditions. It was especially marked in eggs inseminated at 8° C., and did not occur at all in eggs which were fertilized after being kept at 35° C. for one hour. It took place whether or not the fertilization membrane had previously separated to a normal extent, and it was not prevented, and possibly even enhanced, by compressing the eggs moderately under a cover glass.

Movement of the sperm head within the egg

In the exceedingly transparent eggs of the summer urchins *Clypeaster* and *Mespilia,* it is possible, with the phase contrast microscope, to observe the sperm head after it has penetrated the cortex and begun its progress toward the center of the egg. The appearance of the head and middle piece is the same as that outside the egg, but it has been impossible so far to detect the tail with certainty inside the egg at this stage.

Whether the cause of the apparent over-all lability of these eggs lies in a highly fluid state of the hyaline ground-substance, a relative paucity of granular inclusions, or a combination of the two conditions has not been studied. However, with the high dry phase contrast objective, the cytoplasm of the unfertilized egg is seen to consist of small currents of endoplasm flowing at random among undefined stationary regions which seem to consist of loose aggregations of various-sized granules and vesicles exhibiting different degrees of density and refringence.

In such a milieu, the sperm head is distinguished by its being larger than any one of the granular inclusions and often markedly refringent. Under the most favorable optical conditions its elongated triangular shape is clearly visible. However, its detection would be extremely difficult without the spasmodic movements which it performs. Once it has been brought into focus, its progress can be followed for two or three minutes while it moves through the cytoplasm with what appears to be a combination of jerky movements on its own part and the effect of local protoplasmic streaming on the part of the egg. In the region around the entering spermatozoön the random streaming characteristic of the unfertilized egg becomes directed to some extent, with a column of noticeably centripetal flow surrounding the path of the sperm.

Whenever simultaneous observations of the sperm head and tail were possible, it was found that movement of one was always associated with a corresponding movement of the other. In both these species (*Mespilia* and *Clypeaster*) the sperm tail, while relatively inactive, exhibits spasmodic movements from time to time, and as a result of these, the sperm head pushed about among the granules, sometimes causing the displacement of an aggregation two or three times its own diameter. Often it was seen to turn through 90 or more degrees, moving to the side and in one case, going back almost to its entrance point.

This activity continues for a period that roughly corresponds to the time required for engulfment of the sperm tail, although it has not been possible to discover any causal relation between the two, and there is actually evidence against such a conclusion. In half the cases of sperm entrance recorded for *Mespilia,* the complete cessation of movement of the sperm head, followed immediately by the disappearance of its membrane and the appearance of sperm astral rays took place while a sometimes considerable portion of the sperm tail was still visible outside the egg. In a single example of this behavior in *Clypeaster,* the sperm aster appeared at 4′20″ (about the usual time), while half the length of the tail was still extending through and well past the fertilization membrane.

These observations coincide perfectly with the one exceptional case described by Chambers (see Fig. 2). Until a phase contrast study is made of Chambers' material (*Echinarachnius parma*) it will be impossible to determine with certainty whether this situation is actually an exception, or whether it is the rule as it has been found to be in the six other echinoderm species under discussion.

Formation of sperm aster—behavior of middle piece

Whatever factors set the time for the end of the spermatozoan's existence as an independent gamete, it occurs with relative constancy about four minutes after the beginning of fertilization in both the species in which the process is clearly visible. At this time, the outline of the sperm head fades from sight, its refringence is lost, and within 20 to 30 seconds its place is occupied by a hyaline mass without well-defined boundaries, from which small hyaline rays begin to extend into the surrounding cytoplasm.

After the disappearance of the sperm head membrane, the middle piece can be seen lying in the egg cytoplasm close to the hyaline pronuclear mass. As the astral rays elongate and presumably force the pronuclear mass toward the center of the egg by pushing against the egg cortex and/or surface layers (E. L. Chambers, 1939), the middle piece travels with it, near the center of the aster. It appears as a vesicle at the upper limit of the size range of ordinary granular inclusions, containing highly refringent material which appears variously as one or two masses or as a ring of material apposed to the inner surface of the vesicle (probably depending upon the direction from which it is observed as it is pushed about by the movement of the cytoplasmic inclusions surrounding it).

It continues to be recognizable without change through the first cleavage, lying near or even against the membrane of the fusion nucleus in approximately the future equatorial plane (*i.e.*, about equidistant from the two poles of the amphiaster), and taking no detectable part in the various changes which precede cleavage. It is finally carried as an inert cell inclusion into one of the blastomeres when the cell divides.

A complete account of the fate of the *Parechinus miliaris* sperm middle piece, obtained from a study of fixed material, was presented by Meves in 1912 and 1914.

The present phase contrast observations are offered only as confirmation of Meves' painstaking and beautiful work, and as giving such final conclusiveness as comes from following the process in one living cell. Meves carried his observations through the fifth cleavage, at which time he found the still unchanged middle piece in either a macro- or a mesomere of the 32-cell-stage larvae.

Union of sperm with egg pronucleus

In the course of the study of sperm heads within *Mespilia* egg cytoplasm, one case was observed in which the spermatozoan entered in the immediate vicinity of an exceptionally excentric egg pronucleus, and worked its way toward the center of the egg for approximately a minute and a half, until it came to within about 5 μ of the pronuclear membrane. At this juncture it was suddenly carried sideways across the intervening space and held against the pronuclear membrane, its own membrane intact and its tail moving as it was gradually drawn into the egg at the usual rate. The sperm head maintained its integrity of form during this period (ca. 2 min.), at the end of which disintegration of its membrane and appearance of the sperm aster took place as in ordinary cases.

DISCUSSION

Several rather interesting problems have been touched on in the course of this study, such as the actual fate of the sperm tail, especially the exact process by which it is taken into the egg; the nature of the stimulus which sets off the fertilization reaction of the egg; the nature of the forces determining the time at which the spermatozoan terminates its life as an independent unit and becomes an integral part of the fertilized egg; the nature of the attracting forces between the egg and sperm pronucleus; and the nature of the stimulus which directs the course of cytoplasmic streaming in the neighborhood of the entering spermatozoan. Since, however, discussion of most of these questions at this time would involve a preponderance of speculation, and since further study should shed the more reliable light of fact on at least some of them, they are suggested here mainly as inducements to further investigation.

The process by which the sperm tail enters the echinoderm egg may be more involved than a first impression would indicate. In no case does the sperm head penetrate into the cytoplasm more than 20 μ from the entrance point during the three or four minutes while the tail is being engulfed; this is difficult to reconcile with the fact that the length of the tail in these species varies between 40 and 55 μ.

The exceptional case of *Mespilia*, in which the tail was very slow in entering some eggs, contributes only complication to this problem, since, even supposing that the tail remains attached in some way to the sperm pronucleus which takes the place of its head, and is gradually drawn after the synkaryon as it moves to the center of the egg, the radius of the egg minus the radius of the synkaryon is clearly less than the length of the tail, notwithstanding which fact the entire tail eventually disappears within the egg.

Given the apparatus for satisfactory observation of spermatozoa, there are certain conclusions which can be drawn from their behavior. It is obvious that the first sperm to reach the surface of an unfertilized egg is not necessarily the one which finally enters it. It is also accepted as fact that, in echinoderm eggs, any point on the surface is capable of receiving a sperm and setting off the fertilization reaction.

It can then be concluded that some optimal condition of vitality in certain spermatozoa gives them the capacity to effect a local change in the outermost living layer of the egg which sets off a receiving reaction on the part of the egg as a whole.

It seems safe to assume further that the spermatozoan capable of setting off such a reaction has been actively able to penetrate the vitelline membrane of the unfertilized egg in order to reach the living, reactive protoplasm. Direct evidence in favor of such a conclusion is obtained from observation of the fertilization process in the egg of *Ostrea gigas*, which has a relatively resistant vitelline membrane. In order to penetrate this membrane, the round-headed spermatozoan maintains a vigorous rotating attack which continues for from six to ten minutes before connection is established with the underlying cytoplasm. In sea urchins, this connection seems to be made almost instantaneously, or within one or two seconds—at least several hundred times faster than in oysters. Since the difference in the thickness of the vitelline membranes does not seem to be of that order, it is a temptation to place this observation among the evidence in favor of the idea that the sea urchin sperm secretes a substance from the tip of the acrosome (Popa, 1927) which has a locally destructive effect on the vitelline membrane.

As a sequel to the observation of the sperm head being attracted to the egg pronucleus, *Pseudocentrotus* eggs were crushed in artificial Ca-free sea water so that the contents flowed out freely. The pronuclei remained intact and recognizable under these conditions, appearing under the phase contrast oil immersion objective as non-refringent, perfectly spherical and completely empty vesicles. When sperm were added to this suspension, they swam freely about, often adhering temporarily or permanently to one or another of the various cytoplasmic inclusions and sometimes attaching themselves to the egg pronuclei. However, they showed no "preference" for pronuclei over other bodies, and were often observed approaching pronuclei in a random fashion, pushing them about for a while, and wandering off again. While it is not suggested that these conditions imitate those within living egg cytoplasm, the observation is offered for such negative value as it may have.

What can be stated positively from this observation is that the attraction between the egg and sperm pronuclei antedates the appearance of the sperm-astral rays, and that syngamy can take place without their intervention. Moreover, it was found that this pattern of sperm entrance and syngamy, which is exceptional in sea urchins, is the invariable rule in the medusan, *Spirocodon saltatrix* (Dan, 1950). The spermatozoan enters the egg in the immediate vicinity of the egg pronucleus, and syngamy takes place without the formation of a sperm aster, so far as could be determined by the most careful phase contrast observation of these exceedingly transparent eggs.

SUMMARY

1. Phase contrast microscope observation of sperm penetration into the eggs of five species of sea urchins—*Mespilia globulus*, *Clypeaster japonica*, *Strongylocentrotus pulcherrimus*, *Pseudocentrotus depressus*, *Heliocidaris crassispina*—and one starfish—*Asterina pectinifera*—have shown that total engulfment of the spermatozoan including all of the tail, is the rule in these species.

2. In every case the fertilization cone makes its first appearance after contact has been established between the sperm head and the living egg cytoplasm, and persists in normal cases for from 5 to 15 minutes after the beginning of sperm penetra-

tion. Subjection of the eggs to some experimental conditions—heat, cold, nicotine solution—causes abnormal production and persistence of the fertilization cones.

3. Engulfment of the sperm tail in these species is usually complete within 2–4 minutes after the beginning of penetration. No significant change in engulfment time could be induced by exposing the eggs to the abnormal physiological conditions tested—heat, cold, aging, exposure to polyspermy-producing agents.

4. In the two species with the most transparent eggs (*Mespilia* and *Clypeaster*), the movements of the sperm head within the cytoplasm can be clearly observed, from the time it passes the cortex until it becomes quiescent, which approximately coincides with the final engulfment of the tail. Progress of the spermatozoan appears to be effected by a combination of autonomous movement and streaming of the egg cytoplasm.

5. About four minutes after the beginning of fertilization, the clear outline of the sperm head is lost and the sperm aster begins to develop. From this time the middle piece of the spermatozoan can be found lying in the cytoplasm, and traced as it moves together with the sperm aster toward the center of the egg. So far as could be determined, it takes no active part in the first cleavage.

6. In one case a sperm head, which had entered an egg close to the exceptionally excentric egg pronucleus, was attracted to and held motionless against the pronuclear membrane, within about a minute and a half after entering the cytoplasm, while its tail was still largely outside the egg. This, however, produced no variation in the subsequent process of engulfment of the tail and formation of the sperm aster.

LITERATURE CITED

CHAMBERS, E. L., 1939. The movement of the egg pronucleus in relation to the sperm aster in the echinoderm egg. *Jour. Exper. Biol.*, **16**: 409–424.

CHAMBERS, R., 1933. The manner of sperm entry in various marine ova. *Jour. Exper. Biol.*, **10**: 130–141.

KOSTANECKI, K. ET A. WIERZEJSKI, 1896. Ueber das Verhalten der sog. achromatischen Substanzen im befructeten Ei. Nach Beobachtungen an Physa fontinalis. *Arch. f. Mikros. Anat.*, **47**: 309–386.

MEVES, F., 1912. Verfolgung der sogenannten Mittelstückes des Echinidenspermiums im befruchteten Ei bis zum Ende der ersten Furchungsteilung. *Arch. f. Mikros. Anat.*, **80**: 81–123.

MEVES, F., 1914. Verfolgung des Mittelstückes des Echinidenspermiums durch die ersten Zellgenerationen des befruchteten Eies. *Arch. f. Mikros. Anat.*, **85**: 1–8.

MEVES, F., 1915. Ueber den Befruchtungsvorgang bei der Miesmuschel (*Mytilus*). *Arch. f. Mikros. Anat.*, **87**: 47–62.

MOSER, F., 1939. Studies on a cortical layer response to stimulating agents in the Arbacia egg. *Jour. Exp. Zool.*, **80**: 423–445.

MOTOMURA, I., 1941. Materials of the fertilization membrane in the eggs of echinoderms. *Sci. Reports of the Tohoku Imp. Univ.*, 4th Ser., *Biol.*, **16**: 345–363.

POPA, G., 1927. The distribution of substances in the spermatozoan (Arbacia and Nereis) studied by intravitam stains and by stains of lipoids according to the method of Schumacker. *Biol. Bull.*, **52**: 238–257.

RUNNSTRÖM, J., 1948. Further studies on the formation of the fertilization membrane in the sea urchin egg. *Ark. f. Zool.*, **40**: 1–19.

SELENKA, E., 1878. Befruchtung des Eies von Toxopneustes variegatus. Leipzig.

WILSON, E. B., 1895. An atlas of the fertilization and karyokinesis of the ovum. Columbia University Press (Macmillan), New York.

WILSON, E. B., 1925. The cell in development and heredity. Macmillan, New York.

YATSU, N., 1909. Observations on ookinesis in *Cerebratulus lacteus*, Verrill. *Jour. Morph.*, **20**: 353–401.

FERTILIZATION IN THE MEDUSAN, SPIROCODON SALTATRIX

JEAN C. DAN

Tokyo Metropolitan University and Misaki Marine Biological Station

Spirocodon saltatrix, a bell-shaped member of the *Tubularioanthomedusae* between four and eight centimeters in diameter, appears in the inner waters of some of the deeper bays near the Misaki Marine Biological Station in the beginning of January. The young adults carry fully developed eggs and sperm from the time of their first appearance, and during the succeeding three months the animals breed constantly, gradually increasing in size and shifting their habitat from the heads of the bays toward the open sea. At the middle of March most of the animals found are at the upper limit of the size range and contain large quantities of eggs and sperm. There is a brief period during which the gametes are obviously not in optimal condition, after which the medusae completely disappear from the waters near the Station. Nothing has as yet been learned concerning the whereabouts of this species during the remaining nine months of the year.

The embryology of *S. saltatrix* has not been investigated because of the failure so far to rear larvae past the gastrula stage or to collect intermediate stages. A method of inducing spawning was developed by Uchida (1927) and elaborated by Kume (1948). Since these animals spawn soon after nightfall, placing them in the dark in the laboratory causes a copious release of mature gametes within approximately an hour, regardless of the day-night relationship. It has been found that a previous exposure of ten to fifteen minutes to the direct rays of an ordinary lamp improves the reactivity to the stimulus of darkness.

Examination of eggs taken from the ovaries during the morning and early afternoon reveals only immature eggs, whereas spawned eggs are always fully mature. It is thus apparent that the light-darkness change is the stimulus for the beginning of the maturation divisions, and eggs removed from the ovaries of females which have been so stimulated go through the maturation divisions on the microscope stage.

All attempts to fertilize such eggs by adding freshly shed sperm to them have, however, so far failed, as have methods such as mixing separately shed egg and sperm suspensions, or adding sperm suspension to "dry" eggs shed from ovaries cut from animals just before spawning time. It is thus obvious that the eggs are fertilizable for only a very short time after their expulsion from the ovaries, although the specific reason for this has not yet been determined.

In order to obtain eggs in the earliest stages of the fertilization process, the following procedure was adopted: several medusae, after removal from darkness, were placed in sea water in separate containers and observed closely. (Since the males invariably spawn first, it was possible to differentiate between the sexes without undue handling of the animals.) Females just before spawning time were placed either in a container with a shedding male or in sea water containing freshly shed sperm, and watched for the beginning of spawning. Since the spermatozoa also lose their fertilizing capacity after a very brief exposure to sea water, considerable care must be exercised to ensure the presence of freshly shed sperm in the sea water into which the eggs are to be shed. As soon as eggs were observed in the container, a sample was removed to a slide and observed with the phase contrast microscope.

Mature, unfertilized eggs (Fig. 1) are slightly oval with a depression at the animal pole, where the polar bodies have just been extruded, and at the base of which the pronucleus lies in contact with the egg surface. One or both of the polar bodies may sometimes be found still adhering to the polar region, although these are usually lost in the course of shedding and the handling of the eggs prior to observation. There are no enveloping layers or membranes, so far as can be determined, either before or after fertilization.

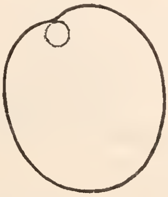


FIGURE 1. Mature unfertilized egg of *Spirocodon saltatrix*. The egg pronucleus lies at the base of a slight depression left after the extrusion of the second polar body.

The fertilizing spermatozoan invariably enters the egg in the immediate vicinity of the egg pronucleus. Further evidence for the existence of some sort of specific attraction on the part of the egg pronucleus for the free-swimming spermatozoa is found in the fact that excess sperm are found only around the animal pole of mature eggs, even in the case of eggs which have been rendered unfertilizable by contact with sea water before the addition of sperm suspension.

Immediately after the penetration of the sperm head into the egg cortex, there develops, around the sperm tail, a tubular structure (Fig. 2a) which gradually increases in both length and diameter for about five minutes (Fig. 2b–e), while the sperm tail, which maintains slight but continuous movement, is steadily being drawn into the egg. The walls of this "fertilization tube" are entirely transparent, and the sperm tail is perfectly visible moving freely within the tube. In several cases a second spermatozoan was found with its head well within the tube.

After attaining approximately the size shown in Figure 2e, the tube begins a course of degeneration, during which it continues to expand in size without, apparently, any increment in material. In fact, there seems to be a chemical dissolution of the structure, as though sea water were an unfavorable medium for its existence. Breaks appear in the walls, and the lashing of the shortening sperm tail causes further disintegration. Especially if there are other spermatozoa around the animal pole of the egg, their movements usually tear the now balloon-shaped structure from its place of attachment, and it gradually becomes more and more attenuated, although fragments of its can sometimes be found after 20–30 minutes.

Complete engulfment of the tail usually requires about 15 minutes. While this is occurring, the egg becomes spherical, and the indentation at the animal pole disappears. The egg pronucleus slowly leaves its original position directly under the surface, moving a short distance toward the center of the egg and showing a marked

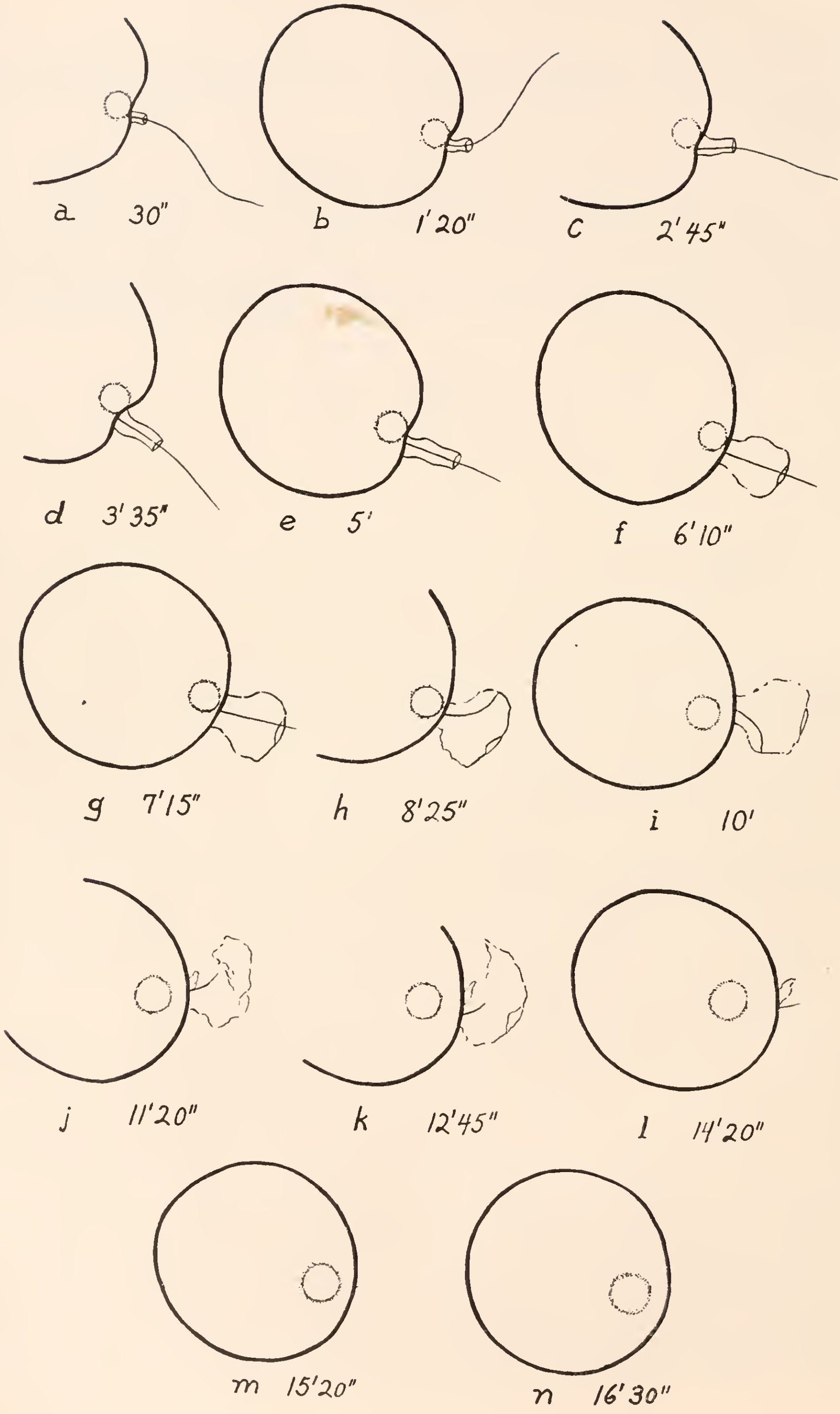

a 30"
b 1'20"
c 2'45"
d 3'35"
e 5'
f 6'10"
g 7'15"
h 8'25"
i 10'
j 11'20"
k 12'45"
l 14'20"
m 15'20"
n 16'30"

FIGURE 2

increase in size. Although the most careful observation of these remarkably transparent eggs failed to reveal any trace of the sperm head after it entered the egg, it seems safe to conjecture that the union of the sperm pronucleus has occurred, without astral activity, between 10 and 14 minutes after the beginning of fertilization. This would account for the increase in nuclear size noted above.

Soon after the stage shown in Figure 2n, the outline of the nucleus disappears entirely, to be followed shortly by the appearance of the cleavage diaster, in the same excentric position previously occupied by the fusion nucleus. For a description of the cleavage pattern, reference is made to Dan and Dan, 1947.

SUMMARY

Eggs of the tubularioanthomedusan, *Spirocodon saltatrix,* which breeds in the neighborhood of the Misaki Station from the beginning of January to the latter part of March, are spawned daily, shortly after nightfall. The change from light to darkness causes the initiation of the maturation divisions, at the completion of which the eggs are shed. Spawning can be induced by darkening in the laboratory.

In the mature eggs, the pronucleus lies in contact with the surface, at the base of a slight depression at the animal pole. These eggs are fertilizable only when spawned into sea water containing freshly shed spermatozoa.

Examination of such eggs with the phase contrast microscope shows that the fertilizing sperm always enters in the immediate vicinity of the egg pronucleus. At the sperm entrance point a tubular structure of transparent substance begins to develop around the sperm tail as soon as the head of the sperm has penetrated the egg. This continues to increase in length and diameter for about five minutes, after which a process of degeneration sets in. At the end of about fifteen minutes the tube has become balloon-shaped and is more or less rapidly disintegrating, as the tip of the sperm tail is finally drawn into the egg.

The egg pronucleus moves a short distance from the periphery toward the center of the egg. A marked increase in size between 10 and 14 minutes after the beginning of fertilization probably indicates the union of the sperm and egg pronuclei, although no astral activity is detectable.

LITERATURE CITED

DAN, K., AND J. C. DAN, 1947. Behavior of the cell surface during cleavage. VIII. On the cleavage of Medusan eggs. *Biol. Bull.,* **93**: 163–188.

KUME, M., 1948. Ovulation and related phenomena in *Spirocodon saltatrix. Collecting and Breeding,* **9**: 270–271.

UCHIDA, T., 1927. Studies on Japanese hydromedusae. I. Anthomedusae. *Jour. Fac. Sci., Tokyo Imp. Univ. Ser. IV,* **1**: 145–242.

FIGURE 2. Camera lucida drawings of sperm entrance in *S. saltatrix.* (Time recorded from beginning of observation.) (a) Immediately after engulfment of sperm head. First appearance of "fertilization tube." (b–e) Increase in length and diameter of fertilization tube, sperm tail gradually being drawn into egg. (f) Beginning of degeneration of fertilization tube, disappearance of polar depression. (f–h) Egg pronucleus beginning to move inward. (i–m) Increase in size probably indicates fusion of egg and sperm pronuclei. Sperm tail completely drawn into egg, remnant of fertilization tube lost. (n) Typically spherical condition of fertilized egg achieved.

OXYGEN CONSUMPTION, BRAIN METABOLISM AND RESPIRATORY MOVEMENTS OF GOLDFISH DURING TEMPERATURE ACCLIMATIZATION, WITH SPECIAL REFERENCE TO LOWERED TEMPERATURES [1]

JOHN A. FREEMAN

Biology Department, Newcomb College of Tulane University, New Orleans

On passing from one temperature to another within the range of viable temperatures, a poikilothermic animal undergoes rapid alterations in its physiological processes. This rapid change is followed by a slower series of changes in the direction of the original state, and a final condition is reached which, in most cases, is intermediate between the original state and that reached shortly after the temperature change. The second, slower series of changes is known as temperature acclimatization.

Among fish, acclimatization may be accompanied by changes in the temperatures compatible with life (Loeb and Wasteneys, 1912; Hathaway, 1927; Fry, Brett and Clawson, 1942; Doudoroff, 1942, 1945; Brett, 1944, 1946), changes in the metabolic rate (Wells, 1935a, 1935b), changes in the rate of respiratory movements (Sumner and Doudoroff, 1938), and changes in the optimum temperatures for sustained swimming (Fry and Hart, 1948).

Though many and extensive studies of temperature acclimatization have been made (Belehradek, 1935), the fundamental processes involved have not been made clear.

On the basis of field observations, Agersborg (1930) suggested that the death of carp (*Cyprinus carpio*) and of gizzard shad (*Dorsoma copedianum*) after passing from water at 28° C. to 30° C. into a lake at about 0° C. is due to respiratory failure. Adrian and Buytendijk (1931) found rhythmic potential changes in the isolated goldfish brain stem which are similar in timing to the respiratory movements. They conclude that the respiratory movements in this fish are manifestations of an inherent rhythm in the central nervous system. In mammals at least, such rhythmic potential changes in brain tissue are dependent upon the metabolic rate of the tissue (Himwich *et al.,* 1939; Hoagland, 1949). In view of this, we may surmise that alteration in the respiratory rhythm results from changes in brain metabolism. If an increase in brain metabolism were found to occur during low temperature acclimatization, it might account for the increase in the rate of respiratory movements and might also show a correlation with the increase in activity and in oxygen consumption which accompany such acclimatization.

Accordingly, the correlation of brain metabolism with total oxygen consumption and with respiratory movements has been examined, first in goldfish previously acclimatized at several different temperatures and then in fish undergoing acclimatization to a lowered temperature.

---

[1] A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Duke University, Durham, N. C. The author wishes to express his appreciation to the University Council on Research of Tulane University for a grant for continuation of this study, some data from which are included in this article.

Materials and Methods

Animals. Goldfish, *Carassius auratus*, obtained from Beldt's Aquarium, St. Louis, Mo., were used throughout the study. The fish ranged in size from 1.5 to 3 inches, exclusive of the caudal fin. On arrival, fish were placed directly in chambers held at the experimental temperatures or into holding tanks until space was available in the experimental chambers. A week or longer was allowed for acclimatization if the previous holding temperature was within 3° of the experimental temperature. Otherwise, the acclimatization period was two weeks or longer.

The fish were fed daily with Beldt's "Best Goldfish Food." Oxygen determinations on the water in which fish were kept indicated that oxygen concentrations were not limiting under the conditions of the experiments (Toryu, 1927; Fry and Hart, 1948).

Temperature control. Temperatures were maintained within 0.1° using thermoregulators. Temperatures below room temperature were maintained by constantly cooling and intermittently heating with thermostatically controlled heaters.

Oxygen consumption of fish. In observations at 37.5° C., the change in oxygen concentration of water passing at a constant rate through a small darkened chamber containing the fish was used in calculations of oxygen consumption. The method is similar to that used by Keys (1930). In observations at other temperatures, the change in oxygen concentration of water in a closed chamber in which the fish were held overnight was used for determinations of oxygen consumption. The method is similar to that used by Ege and Krogh (1915). Corrections were made in both cases using chambers without fish but otherwise treated in a similar manner. Oxygen concentrations were determined by the standard Winkler titration (American Public Health Association, 1946).

Oxygen consumption of brain tissue. Fish were taken from the water and the brains exposed immediately by removal of the roof of the skull. The brain was severed posterior to the vagal lobes, lifted out and blotted briefly on filter paper to remove blood and meninges. Brains from at least two (and usually three) fish were pooled, weighed together to 0.2 mgm., ground in a dry mortar and taken up in phosphate buffer of pH 7.5 prepared by mixing 0.160 *M* KH_2PO_4 and 0.121 *M* $NaHPO_4$.[2] The breis thus prepared were transferred to Warburg flasks with sufficient buffer to bring the volume to 3.00 ml. For CO_2 absorption, 0.20 ml. 10% KOH solution was placed in the central well of each flask along with a piece of folded filter paper. About ten minutes elapsed between the removal of the first fish from water and mounting of the flask and manometer on the Warburg apparatus.

After allowing ten minutes for thermal equilibration, readings were made at ten minute intervals at temperatures other than 0° C.; at 0° C. readings were made at 30-minute intervals. Corrected pressure changes for five intervals were plotted against time on graph paper and the best fitting line drawn "by eye" to obtain a slope for use in calculation of the rate of oxygen utilization.

Rates of opercular movements. Rates of opercular movements were determined by timing fifty movements of each of ten to twenty fish at 12°, 20°, 27° or 37.5° C.

---

[2] The effects of added glucose on brain brei metabolism will be reported elsewhere.

between 7:30 and 9:30 A.M. At 4° C., ten movements were timed on each of four fish on five consecutive days.

RESULTS

Certain aspects of the metabolism of goldfish completely acclimatized to several temperatures were first examined. Specifically, oxygen consumption of the fish and of brain and muscle tissues were measured and the rates of opercular movements were studied. The oxygen consumption of brain tissue from fish acclimatized at two temperatures was studied over the temperature range from 0° to 37.5° C. These data served as the basis of a subsequent study of changes during the course of acclimatization.

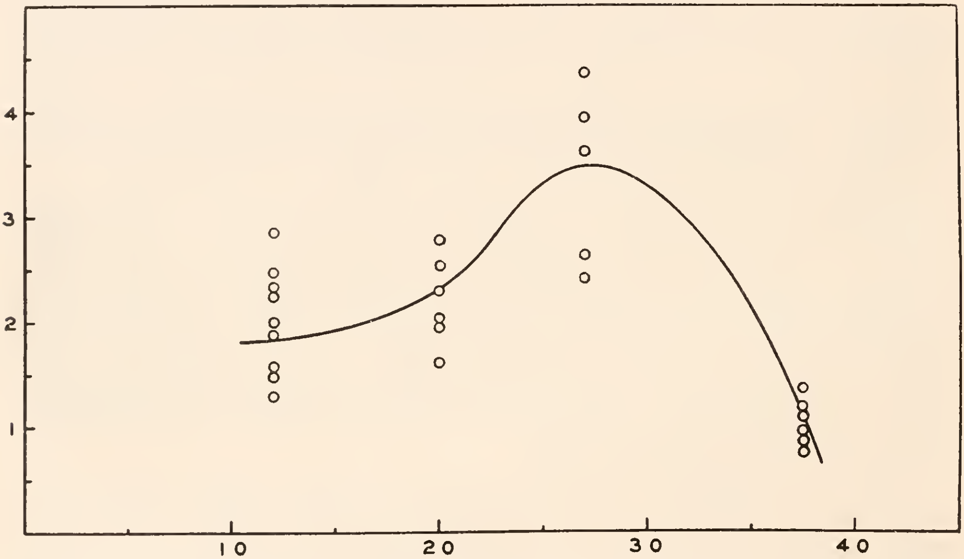


FIGURE 1. Oxygen consumption of goldfish at acclimatization temperatures. Ordinate: oxygen consumption in mgm./kg./min.; abscissa: temperature (C.).

Oxygen consumption of fish at acclimatization temperatures. Data on the oxygen consumption of whole fish at the acclimatization temperatures (Fig. 1) exhibit a maximum at temperatures around 27° C., though there is considerable variation at each temperature studied.

Brain tissue respiration. Brain tissue respiration was studied as possibly correlated with respiratory movements and with total oxygen consumption and overall activity of the fish.

Breis from the brains of fish acclimatized at 12°, 20°, 27°, and 37.5° C. were studied at 18.9° C., a temperature near the center of the range of temperatures tolerated by goldfish. Use of this temperature also avoids the possibility of heat inactivation of brain enzymes. Throughout the range studied, an inverse relation is found between acclimatization temperature and the metabolic activity of brain tissue breis measured at 18.9° C. (Fig. 2). Between 12° and 27° C. the

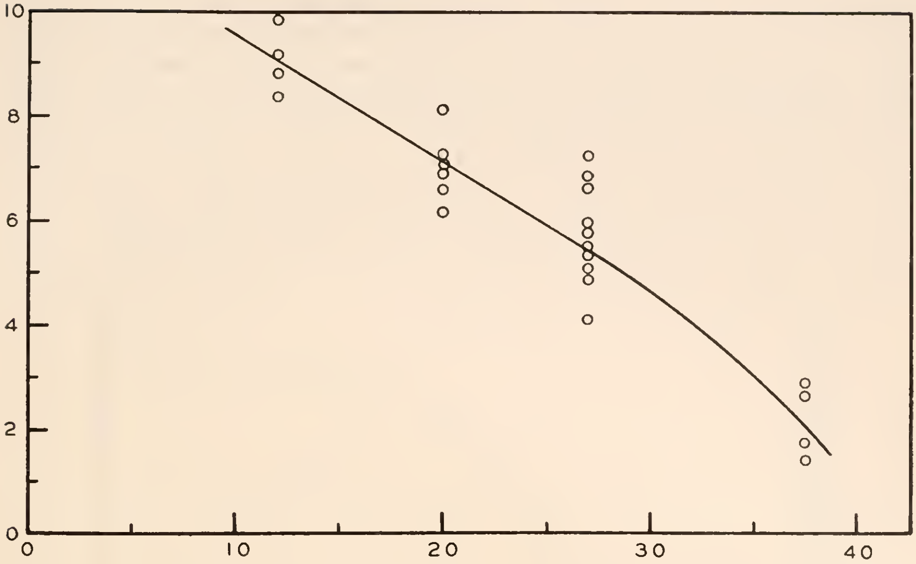


FIGURE 2. Oxygen consumption at 18.9° C. of brain tissue from fish acclimatized at various temperatures. Ordinate: oxygen consumption in mgm./kg. brain tissue/min.; abscissa: acclimatization temperature.

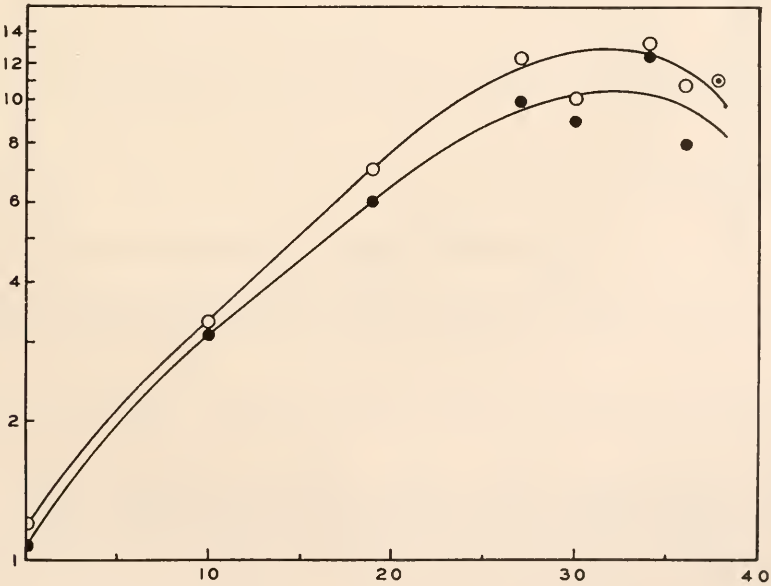


FIGURE 3. Oxygen consumption at various temperatures of brain tissue from fish acclimatized at 20° C. and at 27° C. Acclimatization temperatures: 20° (○); 27° (●). Ordinate: oxygen consumption in mgm./kg. brain/min.; abscissa: temperature.

relationship appears to be a linear one but at higher temperatures the decrease in activity with increasing acclimatization temperature is more marked; and by extrapolation it appears that the activity would be zero for fish acclimatized at about 42° C., a temperature 1° above the maximum upper incipient lethal temperature for young goldfish found by Fry, Brett and Clawson (1942).

The respiration of excised goldfish brain tissue was also studied over the temperature range from 0° to 37.8° C. for fish acclimatized at 20° C. and at 27° C. (Fig. 3). The metabolism of the tissue in each case increased rapidly between 10° C. and 27° C. but was more nearly constant at higher temperatures. At 0°, the rate of oxygen consumption was somewhat lower than would be anticipated from logarithmic curves for the data at 10° to 27° C. Except at 37.8° C., oxygen consumptions were higher for breis of fish acclimatized at 20° than for those acclimatized at 27° C.

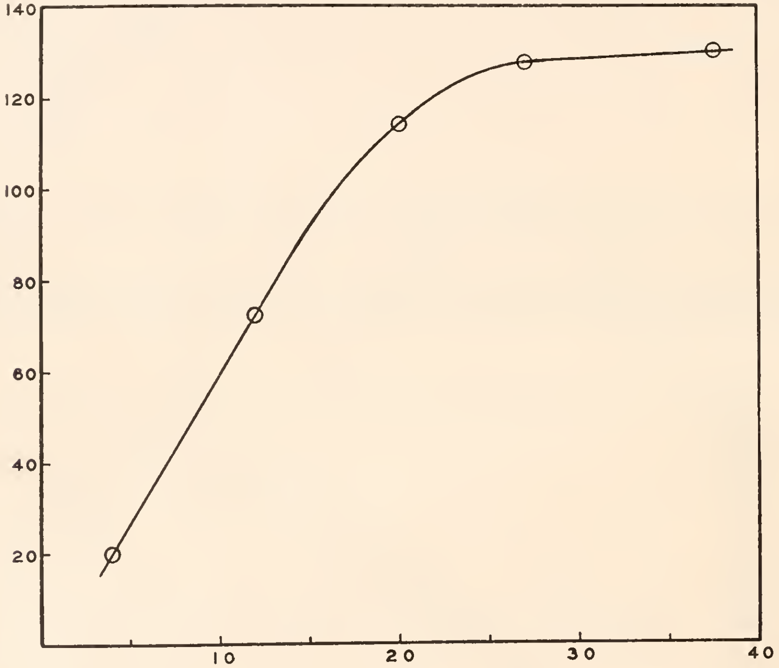


FIGURE 4. Rates of opercular movements of goldfish at various acclimatization temperatures. Ordinate: average opercular movements per minute; abscissa: acclimatization temperature.

Oxygen consumption of muscle tissue. Oxygen consumption of muscle tissue breis from fish acclimatized at 12° and at 27° C. were measured in phosphate buffer at 18.9° C., average values of 1.2 and 1.5 mgm. O_2 per kg. muscle per minute being found, respectively. The difference between these values may not, however, be significant.

Opercular movements at acclimatization temperatures. Rates of opercular movements were studied at acclimatization temperatures 4°, 12°, 20°, 27°, and 37.5° C. as a function which is normal and necessary to the fish and which may be determined with a minimum disturbance to the fish. Furthermore, it may be a function which limits the temperatures at which fish may survive. Between 4° and 20° C. there is a linear relationship between the average rate of opercular movements and the temperature; above 20° C. the rate increases more slowly with temperature and between 27° and 37.5° C. there is little change in the rate of opercular movements (Fig. 4).

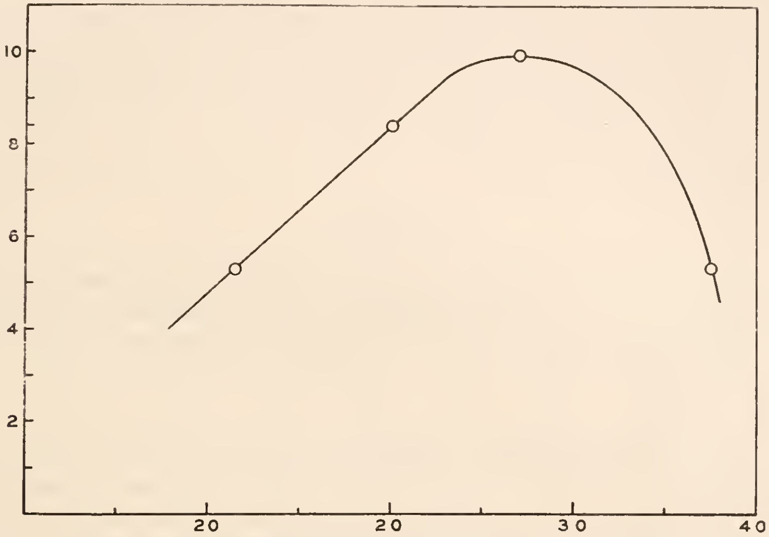


FIGURE 5. Oxygen consumption of goldfish brain brei calculated for acclimatization temperatures. Ordinate: oxygen consumption in mgm./kg. brain/min.; abscissa: acclimatization temperature.

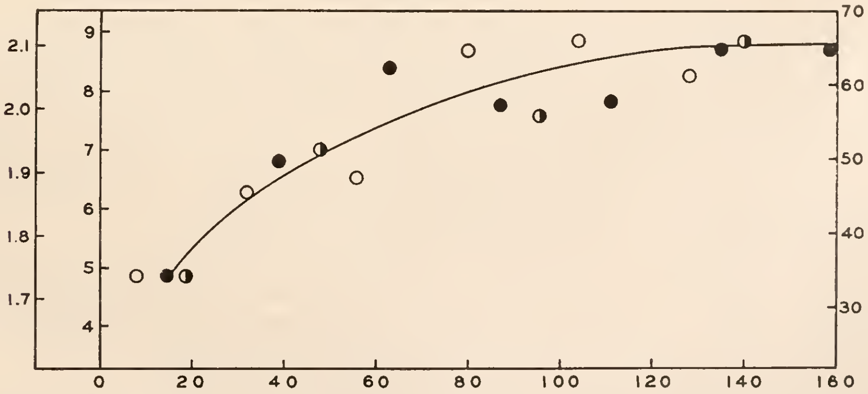


FIGURE 6. Rates of opercular movements, oxygen consumption of fish and of brain tissue during acclimatization of goldfish at 12° C. after previous acclimatization at 27° C. Ordinates (left to right): oxygen consumption of fish in mgm./kg./min. (●); oxygen consumption of brain brei in mgm./kg./min. (◑); and average rate of opercular movements per minute (○); abscissa: hours after transfer from 27° C. to 12° C.

Change of oxygen consumption of fish and of brain tissue and of opercular movement rates during acclimatization. Curves for the rates of opercular movements (Fig. 4), brain respiration at acclimatization temperatures (Fig. 5) and of oxygen consumption of fish at the acclimatization temperatures (Fig. 1) show marked similarities. In order to test these relationships further, the rates at which these change during acclimatization of fish at 12° C., after previously having been acclimatized at 27° C., have been observed. Observations were made at 24 and 48-hour intervals until values were obtained which approximated in each respect those for fish fully acclimatized at 12° C. The results of these observations are presented in Figure 6. The changes in each case occurred rapidly during the first 48 hours after transfer from 27° to 12° C., about 50 per cent of the total change occurring in this period; and the changes were nearly complete in four days.

The rate of change of the opercular rhythm after transfer from 20° to 27° C. was studied with similar results.

Discussion

The changes in brain metabolism, oxygen consumption and in opercular rhythm during acclimatization to lowered temperature are in the direction which might account for the better adaptation of the animals to the low temperatures after acclimatization. The increase in each respect partially offsets the initial effect of the temperature change.

The observations on oxygen consumption of the fish agree with previous observations on the Pacific killifish (*Fundulus parvipinnis*) and on mud suckers (*Gillichthys mirabilis*) by Wells (1935a; 1935b) and, in part, with those on young goldfish acclimatized at various temperatures reported by Fry and Hart (1948). Similar relations between acclimatization temperature and opercular rates have also been reported for the mud sucker by Wells (1935b) and by Sumner and Doudoroff (1938).

Only two reports of studies of the metabolism of fish brain tissue have appeared, that of Fuhrman *et al.* (1944) on the metabolism at graded temperature levels of the brain tissue of the largemouthed bass (*Huro salmoides*) and that of Field and Peiss (1949) on the metabolism of brain tissue from the polar cod (*Boreogadus saida*). These results are similar to those obtained in the present study on the metabolism of brain breis from fish acclimatized at 20° and 27° C. The cod, living at lower temperatures, exhibited higher rates of brain metabolism than the bass; just as in the present study the brain metabolism at a common temperature was highest for fish acclimatized at the lowest temperatures.

When fish respiration and opercular rhythms are compared with rates of brain metabolism calculated for acclimatization temperatures, the depression in brain metabolism at 37.5° C. is found to be intermediate between that for total metabolism and the opercular rhythm. In view of this it is notable that after acclimatization at 37.5° C. the fish are less active in other respects than those at 27° C.

Previous attempts to relate the metabolic changes of the whole organism during acclimatization to the metabolic activity of a single tissue or organ have failed. The present study indicates, however, that there is an inverse relationship between the level of activity of the excised brain tissue and the acclimatization temperature of the goldfish, and that the rate at which brain metabolism changes during temperature acclimatization is of the same order as that for opercular movements and fish oxygen consumption. Further, the oxygen consumption of brain tissue over the range from 0° to 27° C. is very similar to that for goldfish as observed by Ege and Krogh (1915) and by Gardner, King and Powers (1922). In neither case, however, can the direct effect of temperature on muscle account for the differences in whole animal respiration, for there are no significant differences in the metabolism of muscle tissue from fish acclimatized at 27° C. and at 12° C.; and Morales (1943) has shown that respiration of frog muscle tissue changes but little with changes in temperature over this range. It would appear that a major factor in determining the level of oxygen consumption of the fish is the metabolic activity of the brain, acting through its influence on the other tissues of the body.

At the three lower temperatures, 12°, 20°, and 27° C., the relation between the average brain respiration and the average rate of opercular movements is a direct one; the ratios between brain brei respiration calculated for the acclimatization temperatures and the observed rates of opercular movements being constant over this temperature range. There are similarities between the curves for the total oxygen consumption of the fish (Fry, 1947) and of brain metabolism calculated for the acclimatization temperatures. There are also comparable variations in the curves for fish and brain tissue metabolism at temperatures below 10° C., and, as previously mentioned, at temperatures near 37.5° C. Such similarities are especially significant in view of the close correlation between the metabolic activity of brain tissue and its rhythmic activity (Himwich *et al.*, 1939; Hoagland, 1949).

The long-term response of the goldfish to a lowering of the environmental temperature is an increase in metabolism and of general activity above the level of unacclimatized fish at the same temperature, an increase having obvious adaptive advantages. The increase in brain metabolism during low temperature acclimatization may be one of the homeostatic mechanisms making possible the maintenance of normal functioning to a higher degree than would otherwise be possible.

SUMMARY

1. Oxygen consumption of goldfish at acclimatization temperatures increases between 12° C. and 27° C. but is again lower at 37.8° C.

2. When measured at a single temperature, there is an inverse relation between oxygen consumption of brain breis and the acclimatization temperature of fish between 12° and 37.5° C. The level of oxygen consumption of muscle breis, however, does not differ significantly for fish acclimatized at 12° and 27° C.

3. When measured over the range from 0° to 37.8° C., the metabolism of fish brain tissue increases rapidly between 0° and 27°, but at higher temperatures the increase with temperature is less marked and at the highest temperature may be lower again.

4. At temperatures up to 27° C., logarithmic plots of the metabolism of brain tissue and of whole animal respiration are similar in slope.

5. Between 4° and 27° C., the rate of opercular movements at the acclimatization temperatures increases with temperature just as does the rate of brain tissue metabolism calculated for the acclimatization temperatures, the ratios of the two being the same at 12°, 20° and 27° C. Above 27° C., however, the rate of the respiratory rhythm does not rise significantly.

6. The rates at which oxygen consumption of the fish, the opercular rhythm and the brain metabolism change during acclimatization to 12° C. after previous ac-

climatization at 27° C. are the same, approximately 50 per cent of the change taking place in two days. This and other observations are interpreted as indicating that the changes in brain metabolism observed to accompany low temperature acclimatization constitute a homeostatic mechanism making possible the maintenance of more normal functioning than would otherwise be possible at the new low temperature.

LITERATURE CITED

ADRIAN, E. D. AND J. J. J. BUYTENDIJK, 1931. Potential changes in the isolated brain stem of the goldfish. *J. Physiol.*, **71**: 121–135.

AGERSBORG, H. P. K., 1930. The influence of temperature on fish. *Ecol.*, **11**: 136–144.

AMERICAN PUBLIC HEALTH ASSOCIATION, 1946. Standard methods for the examination of water and sewerage. 9th ed. New York.

BELEHRADEK, J., 1935. Temperature and living matter. *Protoplasma Monographien*, **8**: 1–277.

BRETT, J. R., 1944. Some lethal temperature relations of Algonquin Park fishes. *Publ. Ontario Fisheries Research Lab.*, **63**: 1–49.

BRETT, J. R., 1946. Rate of gain of heat-tolerance of goldfish (*Carassius auratus*). *Publ. Ontario Fisheries Research Lab.*, **53**: 5–30.

DOUDOROFF, P., 1942. The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with *Girella nigricans* (Ayres). *Biol. Bull.*, **83**: 219–244.

DOUDOROFF, P., 1945. The resistance and acclimatization of marine fishes to temperature changes. II. Experiments with *Fundulus* and *Atherinops*. *Biol. Bull.*, **88**: 194–206.

EGE, R. AND A. KROGH, 1914. On the relation between the temperature and the respiratory exchange in fishes. *Internat. Revue d. ges. Hydrobiol. u. Hydrog.*, **7**: 48–55.

FIELD, J. AND C. N. PEISS, 1949. Tissue respiration in the polar cod (*Boreogadus saida*) as a function of temperature. *Federation Proc.*, **8**: 81.

FRY, F. E. J., 1947. Effects of the environment on animal activity. *Publ. Ontario Fisheries Research Lab.*, **55**: 5–62.

FRY, F. E. J., J. R. BRETT AND G. H. CLAWSON, 1942. Lethal limits of temperature of young goldfish. *Revue canadienne de Biol.*, **1**: 50–56.

FRY, F. E. J. AND J. S. HART, 1948. The relation of temperature to oxygen consumption in the goldfish. *Biol. Bull.*, **94**: 66–77.

FUHRMAN, F. A., N. F. HOLLINGER, J. M. CRISMON, J. FIELD AND F. W. WEYMOUTH, 1944. The metabolism of the excised brain of the largemouthed bass (*Huro salmoides*) at graded temperature levels. *Physiol. Zool.*, *17*: 42–50.

GARDNER, J. A., G. KING AND E. B. POWERS, 1922. The respiratory exchange in fresh water fish. III. Gold fish. *Biochem. J.*, **16**: 522–529.

HATHAWAY, E. S., 1927. Quantitative study of the changes produced by acclimatization in the tolerance of high temperatures by fishes and amphibians. *Bull. Bur. Fisheries*, **43**: 169–192.

HIMWICH, H. E., Z. HADIDIAN, J. F. FAZEKAS AND H. HOAGLAND, 1939. Cerebral metabolism and electrical activity during insulin hypoglycemia in man. *Amer. J. Physiol.*, **125**: 578–585.

HOAGLAND, H., 1949. Rhythmic behavior of the nervous system. *Science*, **109**: 157–164.

KEYS, A. B., 1930. The measurement of the respiratory exchange of aquatic animals. *Biol. Bull.*, **59**: 187–198.

LOEB, J. AND H. WASTENEYS, 1912. On the adaptation of fish (*Fundulus*) to higher temperatures. *J. Exp. Zool.*, **12**: 543–557.

MORALES, M. F., 1943. Some thermoanalytic studies of organ and whole animal respiration. *J. Gen. Physiol.*, **26**: 381–389.

SUMNER, F. B. AND P. DOUDOROFF, 1938. Some experiments on temperature acclimatization and respiratory metabolism in fishes. *Biol. Bull.*, **74**: 403–429.

TORYU, Y., 1927. The respiratory exchange in *Carassius auratus* and the gaseous exchange in the air bladder. *Tohoku Imp. Univ. Sci. Reports*, Series 3, **4**: 87–96.

WELLS, N. A., 1935a. Variations in the respiratory metabolism of the Pacific killifish *Fundulus parvipinnis* due to size, season and continued constant temperature. *Physiol. Zool.*, **8**: 318–336.

WELLS, N. A., 1935b. Change in rate of respiratory metabolism in a teleost fish induced by acclimatization to high and low temperature. *Biol. Bull.*, **69**: 361–367.

REGENERATION IN AN EARTHWORM, EISENIA FOETIDA (SAVIGNY) 1826. III. REGENERATION FROM SIMULTANEOUS ANTERIOR AND POSTERIOR TRANSECTIONS

G. E. GATES

Colby College, Waterville, Maine

This contribution is the third, and last, in a series presenting results of an attempt to obtain, for one particular species of earthworm, complete characterization of regenerative capacity with reference to exact intersegmental levels. In previous parts (1949, 1950) anterior regeneration by posterior substrates and posterior regeneration by anterior substrates were considered. In this part, anterior and posterior regeneration by fragments with two simultaneously made transections is considered.

Summary of Previous Work

Regeneration by earthworm fragments, following two simultaneous transections, has been studied by several of the investigators cited in parts I–II. Operations were usually made at more or less roughly estimated levels, or at approximate levels, sometimes calculated from the posterior end (*i.e.*, from a segment which may be the 125th to the 75th). On first reading, Morgan (1897), for one set of experiments, appeared to have presented results of regeneration, in *E. foetida,* at definite levels. They are summarized, with some rearrangements and omissions, in Table III. That work was done prior to rediscovery of heteromorphosis, in the Oligochaeta, in 1898–1899, since when no attempt has been made, in any Lumbricid species, to determine the morphological nature of simultaneous regenerates, from two transections, that were not quite obviously recognizable as caudal.

Figure 3 is of a fragment "of 14 old segments, two new ones at anterior end and a few new segments also at posterior end. March 20–April 20." Dates are of 13-segment substrates comprising iii-xv. In the figure the substrate at first appears to comprise only 14 segments but use of magnifying glass shows, on the left side, half of a fifteenth segment (unstippled). No 15-segment substrates were mentioned in the table. Both regenerates, as figured, appear to have had metameric abnormalities. Assuming that the drawing of the anterior regenerate shows an attempt, as it were, at formation of a three-segment regenerate, anterior transection may well have been at 3/4, with posterior at 18/19. Otherwise levels may have been 2/3 and 17/18.

Figure 4 is of a fragment "of 13 old segments, and two new ones at anterior end (replacing 3–5) March 20–April 20." The dates mentioned, according to Morgan's Table, were those of 13-segment fragments comprising iii–xv, as well as of 6, 7, and 8-segment fragments comprising "3, 4, 5–10" segments. As the figure shows thirteen substrate segments, anterior transection is taken to have been at 2/3. The indication in Morgan's Table II that Figures 3 and 4 are of specimens from the "3, 4, 5–10" series is obviously incorrect.

Figure 5, as well as 6 and 7, is, according to Morgan's Table II, of a 14-segment fragment comprising vii–xx. The caption says "13 old segments, and three new ones at anterior end (replacing 7?) April 1–May 13." No 13-segment fragments appear to have had anterior transections at 7/8. Only the first two segments of the figure are stippled (indicative of regenerated segments throughout the plate). The unstippled

TABLE III

Anterior and posterior regeneration, in E. foetida, by fragments from region of i-xxx, according to Morgan (1897 and 1901)

Number of segments	Segments included in substrates	Number of operations	Number of anterior regenerates	Number of posterior regenerates	Morgan's figure no.	Date
3	iii–v	? (a)	+	0		1901 *
4	ii–v	? (a)	+	0		*
8	ii–ix	25	0	0		1897
8	iii–x	50 (b)	1	0 (?)	3, 4 (g)	
10	iv–xiii	10	1	1 (c)		
12	iv–xv	10	1	0		
13	iii–xv	25	2	0		
13	iv–xvi	50 (d)	6	0		
14	vii–xx	55 (e)	8	0 (?)	5, 6, 7 (g)	
16	xv–xxx	26	0	0		
17	iv–xx	15	1	0		
18	iii–xx	26	0	0		
22	ix–xxx	20	0	0		
24	vii–xxx	40 (f)	9	2	8	

According to the text, 81 fragments had the posterior transection at 20/21, but the number according to Morgan's Table was 96.

Omitted above are 25 fragments cut so as to comprise segments "3, 7–15" none of which regenerated, and 25 comprising segments "3, 4, 5–10" two of which regenerated anteriorly (see note b).

* Aside from a statement in a general work, p. 12, no other reference to this experiment has been found.

(a) Number of operations not indicated.

(b) These operations, April 1, according to Morgan's Table, resulted in only one regeneration anteriorly. But the text mentions two pieces, comprising "3–10 segments inclusive" regenerating anteriorly. Presumably one of those was from a March 20 series, omitted from Table above, of 25 animals cut to comprise "3, 4, 5—10" segments. According to Morgan's Table, another fragment of that series also regenerated anteriorly and it could have comprised 6 (v–x), or 7 (iv–x) segments.

(c) A fragment comprising iv–xiii "seemed to have also begun to regenerate at the posterior end" on March 2 (operation Jan. 27) but on March 28 no specimens were found in the pot. On that substrate only, of the 316 (?) with the posterior transection in front of 30/31, was there indication of posterior regeneration, according to Morgan's Table.

(d) Some substrates with regenerates apparently died between examinations.

(e) Of the 25 fragments of March 20 operations, none regenerated. All regenerates were from a second series, of April 1.

(f) Some question as to number of operations on Nov. 9.

(g) In spite of the fact that Morgan s Table and text, except as noted in (c) above, give no indication of posterior regeneration at levels anterior to 30/31, certain figures (camera drawings) and their captions indicate otherwise and accordingly require consideration.

third segment could represent a substrate segment much reduced in size, making 14 substrate segments. Presumably the substrate then comprised segments vii–xx. (Fig. 4 apparently shows an even more marked reduction in size of a substrate segment, in this case the posteriormost.)

Figure 6, according to the caption, is of a fragment of "15 old segments, a few new ones at anterior end (replacing 7) and several new posterior segments." No 15-segment substrates are listed in Morgan's Table. As the figure clearly shows 15 substrate segments, fragments may have comprised segments vii–xxi or vi–xx. The drawing indicates abnormality in the anterior regenerate.

Figure 7 is of a fragment of "13 old segments with five new anterior ones (replacing 7) and a few new posterior segments." The figure shows 14 substrate segments quite clearly, with an unstippled metamere which presumably represents a 15th substrate segment much reduced in size and at first glance apparently part of the posterior regenerate. If the anterior transection was at 6/7 then the posterior transection would have been at 21/22.

Figure 8, according to Table and dates, should be of a 24-segment fragment comprising vii–xxx. According to the caption it is of a 16-segment fragment "with four new anterior segments (replacing 4)" and would then comprise segments v–xx. As the Figure does not show number of segments in fragment, no guess as to original axial location seems feasible. The Figure does show possibility of equimeric regeneration, by a fragment, at an anterior transection as far back as 4/5.

Material, Methods and Nomenclature

In addition to information in corresponding sections of parts I–II (Gates, 1949, 1950) only the following comments are necessary.

Portions of a worm, regardless of size, if with intact buccal or anal region, have been characterized in parts I–II as anterior or posterior substrates respectively. The characterization of "fragment" has been reserved for pieces, regardless of size, with simultaneously made anterior and posterior transections.

Simultaneous, in this article, refers to transections made within a few seconds of each other.

Transections (see Tables IV and V) were made at exactly ten-segment intervals, beginning at 10/11 in one series, at 15/16 in another series; at 15-segment intervals, beginning at 5/6; at 20-segment intervals, beginning, in one series, at 5/6, in another series, at 19/20; and 40-segment intervals, beginning at 35/36. Anterior portions of the first fifteen segments, from one of the series, were transected at 8/9 to give seven-segment fragments, ix–xv, but little or no information was secured from that and other experiments with fragments smaller than ten segments.

Experiments with fragments were conducted from fall to late spring.

In most series, all fragments from the same level, though of different worms, were kept together in one dish. In a few cases, all fragments of one individual were kept together in one dish.

Autotomy, which had been rather annoying in previous work on *Perionyx excavatus* E.Perrier 1872 (Gates, 1927, p. 361) and which may have been an unrecognized source of some of the confusion in Morgan's work on *E. foetida*, was entirely lacking in the author's experiments with anterior and posterior substrates. Two cases of autotomy, with loss of one and three segments, were observed in the work on fragments. As the loss of even one segment results in a piece of different composition and condition from those originally provided by the operation, fragments that had undergone autotomy were excluded. When operated worms were handled as previously described (Gates, 1949a), rather than being kept in dirt or manure and examined less frequently, recognition of autotomy shortly after the event was possible. No autotomy by regenerates was observed. (In some of Morgan's records of 1902, apparently showing, in examinations at two-month intervals, a temporary decrease in number of segments in regenerates, autotomy or amputation in regenerate presumably was involved.)

Morgan (1897) illustrated in his Figure 17, a fragment "regenerating two posterior ends." Both new growths, drawn as tails, appear to be lateral outgrowths such as may be induced by incisions in the nerve cord. No growths were indicated terminally where transections had healed. It seems preferable to reserve the term *regenerate* for structures replacing removed portions and developing only at level of removal.

SUMMARY OF RESULTS

Healing usually was cicatricial. In some fragments the cicatrix, at one or both surfaces, remained imperforate and without externally recognizable change, regardless of lapse of time after operation. In other fragments, at one or both surfaces, and with little or no elevation or protuberance, a cicatrix became characteristically sculptured by formation of a smoothly margined, vertically slit-like aperture that reduced the cicatrix to a narow peripheral band. Often after a variable time, such a sculptured cicatrix slowly became protuberant as a bud, which, with further growth, and metameric differentiation, always became a tail.

Externally recognizable reorganization of substrate segments of the drastic nature later found in *P. excavatus* (Gates, 1941) was not noted. A less drastic form of reorganization involving marked reduction of one or even two substrate segments proximal to the transection, at times to a size only very slightly larger than that of the proximal regenerate segments, was noted in some cases. This type of reorganization involved no change in pigmentation. Some reduction in size charterized all segments of fragments surviving for long periods.

Considerable individual variation as to ability to survive operation and to regenerate was noted. In ten-segment experiments, although three, four, or five fragments of some individuals regenerated, every fragment of other specimens died within the first three days following operation. Several of such individuals were somewhat larger than usual, with especially bright coloration, provided with an unusual amount of chloragogue, and apparently in exceptionally good condition. Fragments of certain individuals survived much longer but without regenerating.

FIGURE 1. Regeneration along main axis of *Eisenia foetida.* Diagram based on regeneration by substrates with single transection only, and on results of following authors. ANTERIOR REGENERATION. *Cephalic:* to 23/24, Gates (part I, p. 135); 35/36, author's interpretation of Morgan's estimated levels, 25/26, 30/31, 34/35, and 50/51 (see Table I, part I, pp. 130–131). *Caudal:* at 18/19, Dimon (see part I, p. 135); 20/21–54/55, Gates (part I, p. 135). POSTERIOR REGENERATION. *Cephalic:* 6/7, author's interpretation of Korschelt's regenerates (see part II, pp. 37 and 43); 34/35, Gates (part II, p. 41). *Caudal:* 20/21, Morgan (see Table II, part II, p. 37), confirmation by Gates (part II, p. 40); no zero level, Gates (part II, p. 41).

Drawing by E. Carpenter, to whom the author's thanks are extended for this assistance.

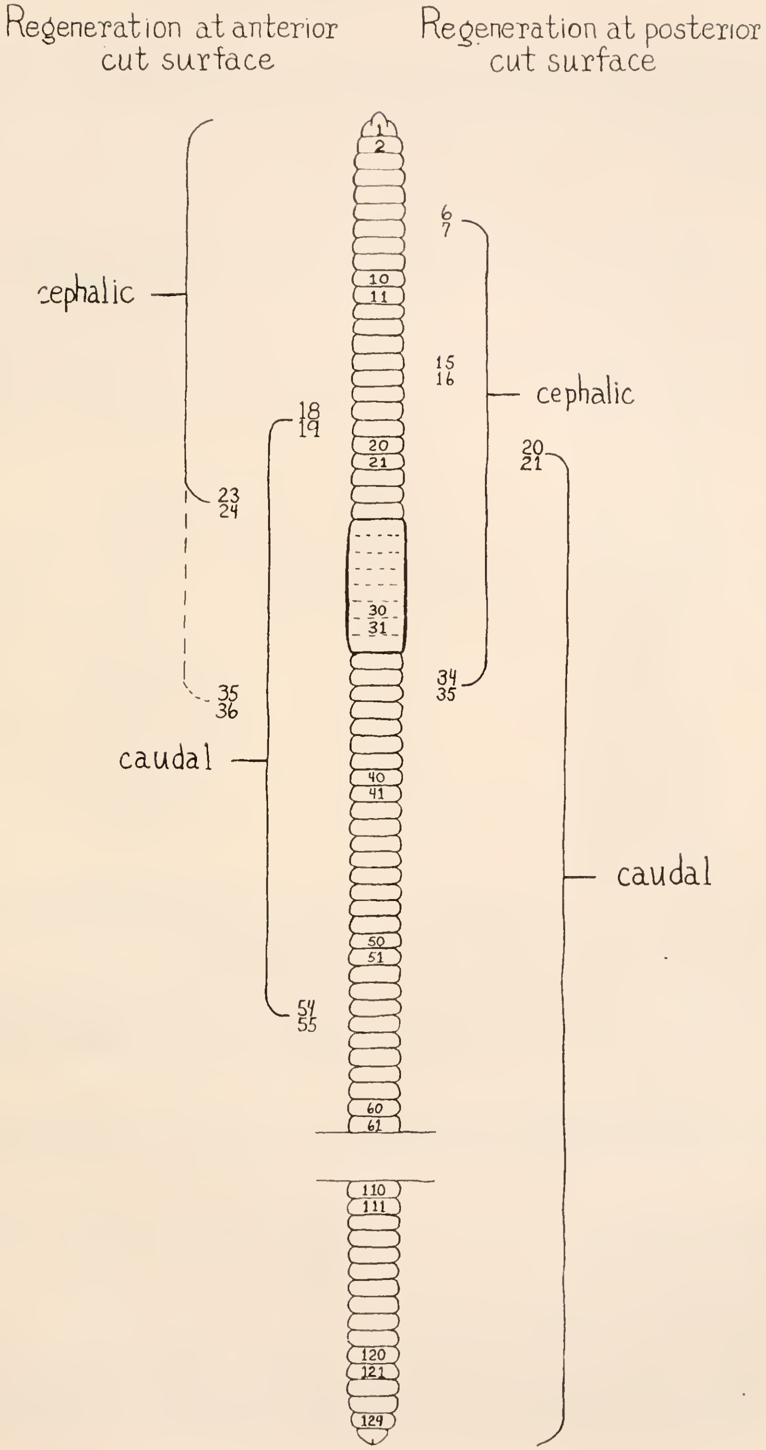

Regeneration at anterior cut surface
Regeneration at posterior cut surface
cephalic
cephalic
caudal
caudal

FIGURE 1

Survival of 10-segment fragments after operation may be longest in region of 65/66–80/81, shorter anteriorly and posteriorly, shortest in region of 10/11–25/26 (see Table IV).

Segmental differentiation, and formation of new segments, continued long after the first month.

TABLE IV

Regeneration and time of survival of ten-segment fragments of E. foetida

Transections at	Survived, days (a)	Number of fragments that regenerated (b)
(...... 8/9) (c)	2–28	0
(......10/11) (c)	1–72	0
(8/9–15/16) (c)	1–27	0
10/11–20/21	1–2	0
15/16–25/26	1–8	0
20/21–30/31	1–79	0
25/26–35/36	1–130	0
30/31–40/41	1–118*	3
35/36–45/46	2–128*	4
40/41–50/51	1–127*	4
45/46–55/56	15–93 *	3
50/51–60/61	10–128*	5
55/56–65/66	8–129*	5
60/61–70/71	15–128*	4
65/66–75/76	75–160*	5
70/71–80/81	52–100*	4
75/76–85/86	6–133*	1
80/81–90/91	16–108*	1
85/86–95/96	10–112†	0
90/91–100/101	8–50	0
95/96–105/106	2–94	0
100/101–110/111	8–96	0
105/106–115/116	1–38	0
110/111–120/121	1–94	0

(a) The first figure of each pair indicates day of first death. The second indicates day of death of last survivor or day of preservation of the set indicated by *. A whole set was preserved if substrates with regenerates had begun to die or to appear moribund.

(b) Occasionally a fragment that had had a regenerate was found to be dead at time of daily examination. Regenerates usually had decayed, sometimes so rapidly that the only trace recognizable was a stain on the paper, and at best segmentation was not distinguishable. None of these are included above but all were from within region of regeneration shown in the Table. Sculptured cicatrixes also are not included.

(c) For comparison, records of two sets of short anterior substrates beginning with i and one set of seven-segment fragments have been included.

† Morgan (1902) had a fragment, presumably of 9–15 segments, that was still alive after 7½ months.

All fragments from regions anterior to 30/31 and behind 90/91, regardless of size, failed to regenerate.

All regenerates from fragments were caudal in nature, regardless of whether produced at anterior or posterior surfaces.

Seventy-six 10-segment fragments, from one set of experiments, presumably survived long enough to produce metamerically differentiated regenerates, but only fifty-six, from a region between 30/31 and 90/91, regenerated. Twelve fragments, from the region between 35/36 and 60/61, regenerated at both surfaces, four at the posterior surface only. Number of segments varied, in homomorphic regenerates, from two to twenty-five when there was no anterior regeneration, from eight to nineteen if there was also anterior regeneration, and in heteromorphic tails from one to fifteen. Total number of segments produced by ten-segment fragments regenerating at both surfaces, varied from nine to thirty-four (1 + 8, 4 + 8, 15 + 15, 15 + 19, etc.). In only one case were numbers of segments in anterior and posterior regenerates equal, and even then the posterior regenerate was obviously the larger. The posterior regenerate always developed first and remained the larger throughout. Occasionally an anterior regenerate did not begin to develop until the second month.

Fragments from the region between 60/61 and 90/91 regenerated only at the posterior transection (Table IV). Absence of regeneration on ten-segment fragments from the region behind 90/91 was independent of position of the fragment, *i.e.*, whether penultimate, antepenultimate, etc.

Table V

Regeneration at posterior surfaces by fragments of E. foetida

Number of segments in fragments	Transections at	Number of regenerates*	Number of segments in regenerates	Average number of segments	Average length in millimeters
10	30/31–40/41	3		6.5	
	40/41–50/51	4		13.	
	50/51–60/61	5		7.6	
	60/61–70/71	4		14.	
	70/71–80/81	4		7.	
	80/81–90/91	1	4	4.	1
15	35/36–50/51	8	13–25	20.6	2.8
	50/51–65/66	7	11–21	15.8	2.2
20	35/36–55/56	9	7–27	20.	3.
	39/40–59/60	5	9–18	13.	1.3
	55/56–75/76	6	8–19	11.5	1.5
40	35/36–75/76	6	9–23	14.6	1.5

* Anally sculptured cicatrixes not counted as regenerates.

Of forty-one surviving 15- and 20-segment fragments, forty regenerated only at the posterior surface (Table V). One 15-segment fragment, however, produced anteriorly, at 35/36, a slender heteromorphic tail marked off into three asetal segments. One worm which had been without food for seventy days was cut into 15-segment fragments. Three survived long enough to regenerate but again at posterior transections only (preservation 85 days after operation, period of starvation 155 days). No retardation of regeneration was observed.

Six forty-segment fragments, with transections at 35/36 and 75/76, survived until preservation at 95 days. Each substrate regenerated at both surfaces though anterior regeneration was unrecognizable until some time after posterior growth was quite obvious to the unaided eye. Number of segments in homomorphic tails varied from 9 to 23 (Table V) and in the always shorter heteromorphic tails from one[1] to eighteen with an average of seven segments, and an average regenerate length of 0.9 mm. Minimum total number of segments in the two regenerates of a single fragment was ten, the maximum, forty-one.

Discussion

A sculptured cicatrix, such as was described above, was not at first considered to be a regenerate. Later on it was discovered that such a cicatrix might grow out into a regenerate that always was caudal and that the aperture of an unprotuberant cicatrix opened into substrate gut. A sculptured but unprotuberant cicatrix accordingly is a small anal region in which a growth zone has not become recognizable and may be considered, even though relatively quite small, a caudal regenerate. For sake of uniformity, the original practice of Morgan was continued throughout. Had anally sculptured cicatrixes been counted as regenerates, percentages of regeneration would, of course, have been higher in the series of this as well as of the two previous contributions. It is probable that an anal regenerate was the structure characterized, prior to rediscovery of heteromorphosis, by Morgan as "not regenerated (mouth present)" (see note f, Table I).

Regenerates, on fragments of ca. 20–22 segments, according to Liebmann (1943, p. 601), showed "all signs of having reached the limit of their growth" at end of one month after operation. The author's results (as well as those from caudal regenerations at single transections, parts I–II) are in agreement with those of Morgan (1902) showing that new segments were produced for several months after operation. When the large regenerative growth zone has been reduced to the very small terminal growth region of normal adult worms, segment formation is quite slow and indications of segment formation may be much more difficult to detect (Gates, 1948).

Absence of cephalic regeneration at anterior transections in region of 30/31–35/36 was unexpected in view of the heteromorphic head obtained at 34/35 (see part II) but is in agreement with the view therein expressed that that level is close to the limit of posterior heteromorphism where head regeneration may be expected only rarely. It is also in accord with the view expressed in part I that Morgan's homomorphic head of five segments at EL50/51 was actually formed at or anterior to 35/36. Failure of fragments from region behind 60/61 to regenerate anteriorly is in agreement with results obtained from normal (unconditioned) posterior substrates which did not regenerate anteriorly at levels behind 54/55.

In a region comprising at least that portion of the axis between 35/36 and 60/61, fragments of ten segments may regenerate at both transections. In view of the ability of forty-segment fragments (35/36–75/76) to regenerate at both transections, it may be expected that fragments of all intermediate sizes also can regenerate at both transections, if from the proper portion of the body, although not observed on the 15- and 20-segment fragments mentioned above.

That portion of the axis behind 60/61, according to the results presented above, comprises two regions; an anterior in which fragments regenerate only posteriorly

---

[1] An anal region, larger than, and more protuberant than the merely sculptured cicatrix mentioned above, if without indication of segmental differentiation, was counted as a single segment.

and a terminal in which fragments do not regenerate at all. For that portion of the axis in front of 30/31 we are still dependent upon the data of Morgan's experiments discussed in the notes to Table III. Fragments with posterior transection at or in front of 15/16 regenerated at only one transection, the anterior. This suggests possibility of a region with regenerative potentiality the reverse of that prevailing in 60/61–90/91. However, since fragments of as few as three or four segments (at least if comprising iii-v or ii-v) replaced the lost anterior segments, an anterior region, corresponding in reverse to that of no regeneration behind 90/91, would appear to be so small as to be only theoretically recognizable.

In the region behind 60/61, the single regenerate of a fragment is caudal, as in posterior regeneration by anterior substrates. Even in absence of definite information as to the nature of Morgan's single regenerates from the region in front of 15/16, there is no reason for suspecting that they are not cephalic, as in case of anterior regenerates from single transections in that region. This also is the reverse of what takes place in 60/61–90/91.

Some of Morgan's fragments from the region in front of 30/31 had two regenerates. The author's experience with other species, as well as with *E. foetida,* enables a suggestion that the small posterior regenerates shown in two of Morgan's Figures (3 and 7) were cephalic, growth inhibited before completion of metameric differentiation and buccal sculpturing. Such heteromorphic posterior regeneration, at the presumed levels 17/18 or 18/19 (as well as at 21/22) is in agreement with the regenerative potentiality indicated in part II from data of regeneration at single transections. Accordingly there appears to be an anterior region, corresponding in reverse to that of 35/36–60/61, where both regenerates of a fragment are the same, cephalic. The posterior limit of that region at present would appear to be set at 19/20 by the ability of anterior substrates to regenerate a homomorphic tail at 20/21.

The posterior regenerate in Morgan's Figure 6 (1897) is clearly caudal. The posterior transection of that substrate appears to have been at or near 21/22 which is within the region of bipotential regenerative capacity. If wholly within that region fragments such as Korschelt's (1897), according to results of regeneration from single transections, would appear to have the following possibilities; of forming a head and tail at proper anterior and posterior transections, a head at both transections, a tail at both transections, a head posteriorly and a tail anteriorly, a head or a tail anteriorly but no regenerate posteriorly, a head or a tail posteriorly but no regenerate anteriorly. Korschelt's fragments from this region probably had tail regenerates posteriorly. Whether the shorter anterior regenerates were caudal or cephalic was not indicated and how many of the possibilities just listed are actually realizable remains to be determined.

No evidence has yet been discovered to indicate that in *E. foetida* simultaneous regeneration at another transection is able to affect morphological nature of a regenerate, as has been found in an Oriental species (Gates, 1941, pp. 168–171). In this connection may be mentioned the fact that in the former, one regenerate always seems to be much slower in beginning to grow.

The chloragogue of 5–6 segments, in *E. foetida,* is depleted, merely by healing at a single transection, while the chloragogue of 10–15 further segments is required for posterior regeneration, according to Liebmann (1943). Presumably then the chloragogue available in ten-segment fragments should have been consumed merely by a double cicatrization. Yet as many as 34 segments (in anterior and posterior regenerates together) were produced by a ten-segment fragment. Even at 50/51 and 70/71 ten-segment fragments may produce, on the average, about as many metameres as forty-segment fragments produce at 75/76. These results seem difficult to reconcile with the thesis of proportionality between available chloragogue and segmental constitution of regenerate. A somewhat similar difficulty resulted in Liebmann's suggestion that chloragogue of the regenerating tail provided the balance of material needed. However, regeneration by fragments, after more than two months depletion of chloragogue by starvation, also indicates other sources than chloragogue are available to provide for growth of the regenerates.

Summary

Regenerates from fragments were all caudal and were obtained from a region behind 30/31. Ten-segment fragments regenerated at both transections in the region between 35/36 and 60/61, at posterior transection only in the region between 60/61 and 90/91, not at all behind 90/91; producing as many segments, in homomorphic tails, as 40-segment fragments. The latter, at 35/36–75/76, regenerated at both surfaces. Heteromorphic regenerates developed later, were always smaller, and almost always differentiated fewer segments than homomorphic tails. Starvation did not inhibit regeneration posteriorly by 15-segment fragments.

General Discussion

Dr. Thomas Williams (1851, p. 247) once declared, "On the authority of hundreds of observations laboriously repeated at every season of the year . . . there is not one word of truth" in previous reports of regeneration by earthworms. That, according to Baird (in Johnston, 1865, p. 321) was, "with all due deference, the language of a froward tongue, and unlike that with which a prudent man dealeth with knowledge." However, the Williams dictum did shortly result in further demonstration of the ability of earthworms to regenerate.

It is now known that several species of earthworms may be found in the manure-pile habitat from which *E. foetida* has so often been obtained and evidence is available elsewhere (Gates, 1949b) that some of them at least may have been confused with *E. foetida*. Attention also has been called (note d, Table I) to specific indications that Morgan may have had individuals of such species in some of his experiments.

Nevertheless, when unable to provide confirmation from his own experiments, the present author has followed the practice of accepting provisionally unusual results of others, if they can be fitted into a general pattern. This seems justifiable in view of the fact that individual variation, as to regeneration and morphological nature of regenerate, is possible even in identical experimental conditions, and especially since the same procedures have seldom been used by any two investigators. Some procedural differences may have had more significance than has been realized. Thus, for instance, Liebmann (1942, p. 153) found that *E. foetida* regenerated "incomparably quicker" in earth than in moist paper or linen. However, at 5/6, and in earth, his worms still had, on the twelfth day, only a bud of half-segment width. By that time the author's worms, in moistened paper, already had metamerically differentiated, equimeric, feeding head-regenerates, at least at 2/3 and 3/4.

The anteroposterior axis of *E. foetida*, in recent years, has been demarcated, more or less definitely but variously, according to regenerative capacity. Crowell (1937) marked off at 35/36, two regions: an anterior in which there is no posterior regeneration, and a posterior in which all regeneration, regardless of direction, is caudal. Avel (1937, 1938) recognized three regions: a head regenerating region ending at 20/21, a middle region of no regenerative capacity, and a posterior region in which anterior regeneration, possible only in a limited number of cases, is caudal and heteromorphic. The boundary between the two posterior regions was placed, rather indefinitely, at 35/36 or 40/41 (1937, p. 28), 30/31 or 40/41 (1938, p. 19). Liebmann (1943) recognized but two regions: one anterior to 20/21 in which regeneration is cephalic, and one posteriorly in which regeneration is caudal.

One factor common to each of those schemes is omission of important data long since available, and in particular with reference to posterior heteromorphosis. Such data, together with that provided in the present series of contributions, show that pattern of regenerative capacity in *E. foetida* is more complicated that has hitherto been thought. An attempt to illustrate the pattern diagrammatically has been made in Figure 1. The various boundaries must, however, be understood to be located only provisionally, and based in part on the author's interpretation of results of other investigators.

Reference to Figure 1 will show an anterior region extending back to and including 17/18 in which regeneration, regardless of direction, is cephalic only. In the anterior part of this region, *i.e.*, in front of 6/7, cephalic regeneration seems to be possible only in one direction—anteriorly. In the posterior portion, *i.e.*, from 6/7 to 17/18 inclusive, regeneration may take place in either direction.

The region from 20/21 to 34/35 (± ?) inclusive has a bipotential capacity, as a regenerate, in either direction; may be either a head or a tail. At levels 18/19 and 19/20 regenerative capacity can also be called bipotential. However, present location of two boundaries indicates a one directional restriction on the bipotentiality, *i.e.*, posterior regeneration at 18/19 and 19/20 restricted to heads, but both heads and tails regenerated anteriorily from the same levels.

Behind 34/35 there is another region in which all regeneration is the same, but in this case caudal, and again regardless of direction. This region also can be subdivided into two parts: a more distal portion—posterior to 54/55, in which regeneration apparently takes place in only one direction—posteriorly, and the remaining portion in which a tail may regenerate in either direction. A sort of overall axial symmetry is thus provided by this reversal of regenerative capacity posteriorly. The symmetry is imperfect, however, as the posterior region and both of its sections are much longer than the anterior region and its sections. This greater length apparently provides a section in which fragments theoretically long enough to regenerate actually appear to be unable to do so.

Unless such anteroposterior symmetry is lacking within the region of bipotentiality there should be therein a posterior subregion of one directional restriction the reverse of that apparently characteristic of an anterior subregion comprising 18/19–19/20.

The morphological nature of a regenerate, in *E. foetida*, is controlled by level, according to Crowell (1937); by mesodermal tissues of the ventral half of the body wall according to Avel (1932); by polarized coelomic bodies composed of aggregates of eleocytes (later called trephocytes) derived from the chloragogue, according to Liebmann (1942–1946). Avel's evidence was provided by several series of unusual and interesting operations but in part from negative results obtained in a region supposedly without regenerative capacity. Liebmann's hypotheses, which have been commented on above and in previous parts, do not explain what organizes the same eleocytes, in one portion of the axis into a head regulating aggregate, and in another portion of the body into a tail regulating aggregate.

In partial agreement with Crowell, level does appear to be of considerable morphogenetic importance. However, bipotential regenerative capacity at numerous levels, in identical external experimental conditions, indicate some further factor or factors should be sought.

Previous discussions of regeneration in earthworms usually have involved an assumption, implied or more or less definitely stated, that pattern of regenerative capacity is identical, not only in species of the same family but even in earthworms in general. Part I showed that the regenerative pattern of the Lumbricid *E. foetida* differs in one very important aspect, inability to regenerate gonads, from patterns of such species of other families as have been studied. Little information as to segmental constitution and morphological nature of regenerates at definite levels is available for other species of the rather small family of the Lumbricidae. Nevertheless, in view of past assumptions as to uniformity of regenerative pattern, certain indications to the contrary are noteworthy.

In *Allolobophora terrestris* (Savigny) 1826, posterior substrates as short as LI4S may regenerate anteriorly (Korschelt, 1897, p. 80). In that species accordingly, the region of anterior heteromorphism now appears to be very much longer, both absolutely and relatively, than in *E. foetida*.

No records have been found of tail regeneration in *Lumbricus terrestris* L. At present it seems necessary to assume that this species is unable to regenerate a tail, at least in the way in which *E. foetida* and many other species do. Yet, on a reversed graft, Ruttloff (1908) obtained a homomorphic tail regenerate at 7/8, a level well in front of any at which there is reason to suspect a tail might regenerate in *E. foetida*.

Segment number may even provide indications as to differences in pattern. Crowell determined boundaries of his morphogenetic regions by data in part from Morgan's experiments on *E. foetida* and in part from his own work on *Allolobophora caliginosa* (Savigny) 1826. His characterizations of regenerative capacity, apparently, were regarded as equally applicable to both species. Recorded maximal segment numbers for those species are 125 and 250, respectively. Accordingly, while level 25/26 has the same numerical value in both species, it is one fifth of the maximum distance toward the posterior end in *E. foetida* but only one tenth in *A. caliginosa*.

In contrast to all such indications is evidence suggesting one contrary trend. In the Glossoscolecid *Criodrilus lacuum* Hoffmeister 1845, Tirala (1912, p. 527) found that regeneration took place "mit Schnelligkeit und Exaktheit" after six months starvation. In the Lumbricid *E. foetida,* as has been shown above and in previous parts, starvation for two or more months does not prevent regeneration. In at least two species of the Megascolecid genus *Perionyx,* the author has found that starvation of a month and more does not inhibit regeneration. This characteristic alone now seems to be common to the regenerative patterns of different families.

In earthworms, it was long thought that " the exposed surface tends to regenerate all the structures that lie in front of or behind it in the normal worm" (Morgan, 1897, p. 582). Even a small fragment was supposed to have ability to restore all missing segments so as to take again its proper position on the anteroposterior axis (Korschelt, 1897). In *E. foetida* it now appears that a posterior substrate can replace completely the excised portion only if the transection is at or in front of 8/9, although a hypermeric regenerate of nine segments (+ 1, see part I) suggests that further study may enable the level to be placed at 9/10. While head formation at levels behind 13/14 may enable continued existence, reproduction, in absence of ability to regenerate or reorganize gonads, would be impossible.

An anterior substrate can completely reform the excised portion only if the transection is at or behind 20/21 and ordinarily only by a combination of regenerative and normal growth. Presumably then, in *E. foetida,* only fragments having anterior transection at or in front of 8/9 and posterior transection at or behind 20/21 would have any possibility of completely replacing lost portions and again occupying the same location along the anteroposterior axis as before amputation. Other fragments may regenerate but in many cases will be doomed to die of starvation or constipation.

Literature Cited

Avel, M., 1932. Le pouvoir régénérateur de moitiés dorsale et ventrale de la paroi du corps, dans la région céphalique. *C. R. Ac. Sci. Paris,* **194**: 2334–2335.

Avel, M., 1937. Titres et Travaux scientifiques de Marcel Avel. Bordeaux.

Avel, M., 1938. Expériences sur l'origine du système nerveux central dans la régénération de la tête chez les lombriciens. *Trav. Sta. Zool. Wimereux, Lille,* **13**: 17–25.

Crowell, P. S., 1937. Factors affecting regeneration in the earthworm. *Jour. Exp. Zool.,* **76**: 1–33.

Gates, G. E., 1927. Regeneration in a tropical earthworm *Perionyx excavatus* E. Perr. *Biol. Bull.,* **53**: 351–364.

Gates, G. E., 1941. Further notes on regeneration in a tropical earthworm, *Perionyx excavatus* E. Perrier 1872. *Jour. Exp. Zool.,* **88**: 161–185.

Gates, G. E., 1948. On segment formation in normal and regenerative growth of earthworms. *Growth,* **12**: 165–180.

Gates, G. E., 1949a. Regeneration in an earthworm, *Eisenia foetida* (Savigny) 1826. I. Anterior regeneration. *Biol. Bull.,* **96**: 129–139.

Gates, G. E., 1949b. Miscellanea Megadrilogica. *Am. Nat.,* **83**: 139–152.

Gates, G. E., 1950. Regeneration in an earthworm, *Eisenia foetida* (Savigny) 1826. II. Posterior regeneration. *Biol. Bull.,* **98**: 36–45.

Johnston, G., 1865. A catalogue of the British non-parasitic worms in the collection of the British Museum. London.

Korschelt, E., 1897. Ueber das Regenerationsvermögen der Regenwurmer. *Sitzber. Ges. Bef. Naturwiss. Marburg,* **1897**, 73–105.

Liebmann, E., 1942. The role of the chloragogue in regeneration of *Eisenia foetida* (Sav.) *Jour. Morph.,* **70**: 151–187.

Liebmann, E., 1943. New light on regeneration of *Eisenia foetida* (Sav.). *Jour. Morph.,* **73**: 583–610.

Liebmann, E., 1945. Experimental study on the role of the trephocytes in regeneration of *Eisenia foetida* (Sav.). *Growth,* **9**: 43–54.

Liebmann, E., 1946. On trephocytes and trephocytosis; a study on the role of leucocytes in nutrition and growth. *Growth,* **10**: 291–329.

MORGAN, T. H., 1897. Regeneration in *Allolobophora foetida*. *Arch. Entwickmech. Org.*, **5**: 570–586.

MORGAN, T. H., 1901. Regeneration. Columbia Univ. Biol. Series, 7.

MORGAN, T. H., 1902. Experimental studies of the internal factors of regeneration in the earthworm. *Arch. Entwickmech. Org.*, **14**: 562–591.

RUTTLOFF, C., 1908. Transplantationsversuche an Lumbriciden. *Arch. Entwickmech. Org.*, **25**: 451–491.

TIRALA, L. G. M., 1912. Regeneration und Transplantation bei *Criodrilus*. *Arch. Entwickmech. Org.*, **35**: 523–554.

WILLIAMS, T., 1852. Report on the British Annelida. *Rep. 21'st Meeting Brit. Ass. Adv. Sci.*, **1851**: 159–272.

ADAPTIVE CHANGES IN THE CHLORIDE CELLS OF ANGUILLA ROSTRATA

HERBERT C. GETMAN[1]

Arnold Biological Laboratory, Brown University and The Marine Biological Laboratory

The production of hypotonic urine in both marine and fresh water teleosts, together with differences in ion content of the urine which were not accounted for by renal excretion, led Smith (1930) to consider other possible sources of salt elimination. He postulated the gill to be the site of extra-renal chloride excretion. The following year, Keys (1931), through use of a heart-gill perfusion system, demonstrated the excretion of chloride by the gill of the eel, *Anguilla vulgaris*. Histological examination of the epithelium of the eel gill filament by Keys and Willmer (1932) revealed an acidophilic, non-mucous, glandular-appearing cell which they suggested might be responsible for chloride excretion. Recently, Copeland (1948), using *Fundulus heteroclitus*, presented evidence of cytological changes during salinity adaptation. Copeland stated that the presence of a clear vesicle at the free surface of the cell in animals adapted to salt water was indicative of chloride excretion. Pettengill and Copeland (1948) noted the marked difference in alkaline phosphatase activity between sea and fresh water adapted Fundulus and suggested this as possible evidence for the excretory and absorptive functions, respectively.

In the meantime, Krogh (1937), instead of studying excretion as previous investigators had done, measured the ability of several fresh water teleosts to absorb chloride from dilute saline solutions. Of the fish studied by Krogh, the eel was the only species to show no evidence of active absorption.

In extending the study of a possible chloride excreting cell in the gill, it was decided to choose not only another euryhaline teleost, but one which has been the subject of previous histological and physiological investigation. Anguilla fitted these two conditions. The considerable work of Keys and his associates in observing the physiological processes operating in the eel during adaptation, and the interesting observation by Krogh that the eel gill was unique in not absorbing chloride, let to this cytological study of the eel chloride cell during adaptation to fresh and salt water.

MATERIALS AND METHODS

The experimental work was divided into two sections: a) 4 eels (*Anguilla rostrata*) were adapted to sea water and 3 to fresh water for 3 to 5 days and then killed for a record of the normal picture in those environments; b) 46 eels were divided into two groups and adapted to sea or fresh water for at least 48 hours; they were then transferred to the opposite solution for varying periods—2, 6, 15 or 24 hours—before killing them.

---

[1] The material reported here is taken from a thesis submitted in partial fulfillment of the requirements for the degree of Master of Arts in Biology in the Graduate School of Brown University.

Part of the animals were experimented upon at Brown University. They were kept in sinks of running tap water or 5 gallon jugs of aerated sea water. The remainder of the eels, investigated at the Marine Biological Laboratory, Woods Hole, Massachusetts, were kept in running sea or tap water aquaria. None of the animals was fed after they were caught.

The eels were killed by beheading and pieces of the gill were fixed for demonstration of Golgi material and mitochondria. The Golgi technique was slightly modified from Ludford's (1926). Fixation was done in Mann-Kopsch solution (2 parts mercuric chloride saturated in 0.5% $CaCl_2$, 1 part 2% osmium tetroxide) for 12–18 hours. The tissue was then washed in distilled water for an hour and postosmicated 3 days at 37° C., followed by an additional day of incubation in distilled water to remove residual tetroxide. When necessary, slides were differentiated in very dilute hydrogen peroxide. For mitochondria the fixation was in Regauds fluid followed by postchromation, then by staining in Altmann's acid fuchsin. The schedule was as described for Fundulus material (Copeland, 1948) except that 1% methyl green was used for differentiation.

RESULTS

General morphology. The chloride cells of *A. rostrata* are localized in the afferent arterial edge and along the sides of the filaments. They are interspersed among the mucous cells along the edge of the filament and are closely packed together in the filament epithelium between the bases of the respiratory platelets. The latter differs from that in *A. vulgaris,* where the localization is found to extend out on the respiratory platelets (Keys and Willmer, 1932).

The shape of the cells varies with their location. Those at the base of the platelets, where the epithelium is limited in thickness by underlying cartilage, are round

---

FIGURE 1. Sea water adapted 5 days. Mitochondrial preparation in the region of the afferent artery. Note the clefts in the epithelial surface. At the base of one of these clefts a pit typical of sea water adaptation is visible at the free surface of the elongated chloride cell. On either side of it are oval mucous cells.

FIGURE 2. Sea water adapted 15 hours. Mitochondrial preparation showing cells along the cartilage at the base of a platelet. Compare the shape of these cells with those located along the afferent artery in Figures 1 and 4. These cells are typical of those along the cartilage, which rarely show a pit but often have a clear area of cytoplasm at the surface extending to the supranuclear region. Cartilage is below; section of an adjacent respiratory platelet is above.

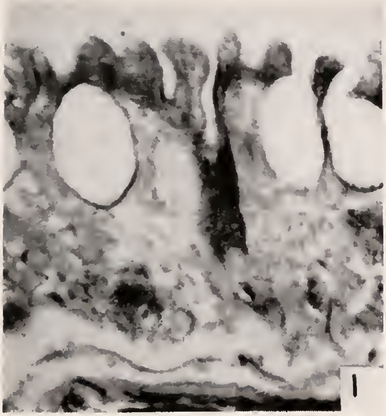
FIGURE 3. Fresh water adaptation 5 days. Mitochondrial preparation in the region of the afferent artery. The chloride cell is less shrunken than those adapted to sea water and the mitochondria are more dispersed.

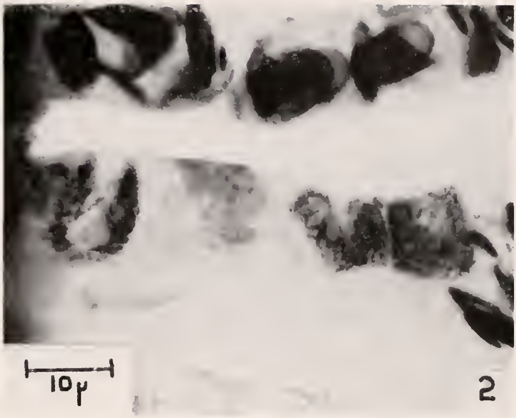
FIGURE 4. Sea water adaptation 15 hours. Mitochondrial preparation of the region along the afferent artery. Note the well defined pit and the densely packed mitochondria.

FIGURE 5. Fresh water adaptation 5 days. Mitochondrial preparation. An example of two fresh water adapted cells along the afferent artery which show definite pits. Compare with Figures 1 and 4 to see that these cells differ from those adapted to sea water in shape and diffuseness of mitochondria.

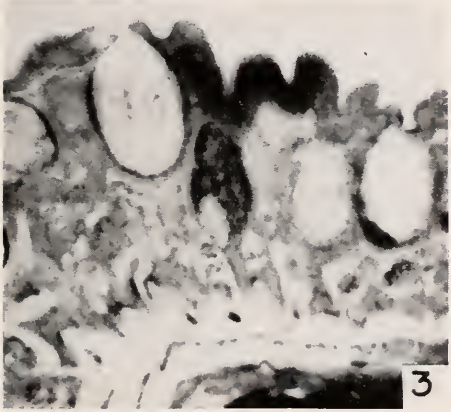
FIGURE 6. Sea water adaptation 15 hours. Osmiophilic preparation of the region along the cartilage. At the left can be seen a row of Golgi bodies. At the right, two cells possess varying amounts of granular osmiophilia. In one of them, a band of Golgi bodies is visible just above the nucleus.

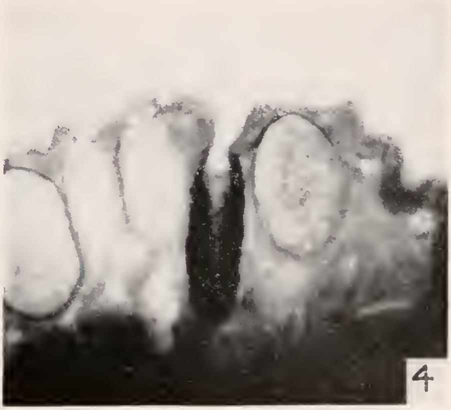
Magnification: all Figures to shown scale

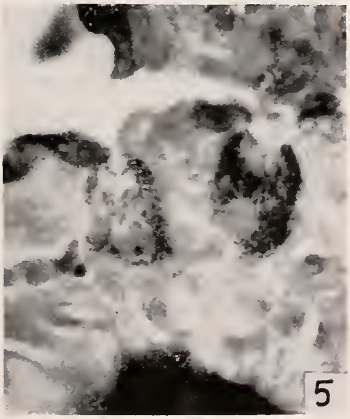

1

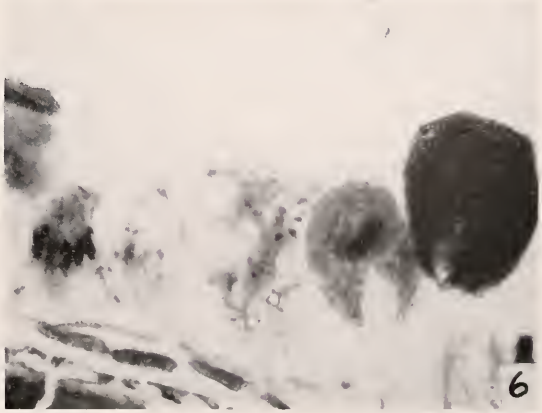


2


3


4


5


6

Figures 1–6

or oval (Fig. 2). The cells in the free edge of the filament are more spindle shaped, often being 3 to 5 times as long as they are wide (Fig. 1). Both variations usually touch the basement membrane and thus are close to blood flowing from the afferent artery to the respiratory platelet.

Completely adapted cell. The cells also vary in shape with adaptation. When sea water adapted, the spindle shaped cells immediately about the afferent artery are quite narrow and irregular in shape (Fig. 1). In fresh water adaptation, they become more plump and oval (Figs. 3 and 5).

In sea water adapted eels, the majority of the long cells adjacent to the afferent artery possess narrow pits (Figs. 1 and 4). Nevertheless, in this species the presence or absence of pits at the distal end of the cells apparently is not an absolute indication of adaptation. In fresh water, although most of the pits disappear, an occasional one can still be seen (Fig. 5). The short, spheroidal cells at the bases of the respiratory platelets rarely show pits under any condition.

The sea water adapted chloride cells possess a dense mass of closely packed mitochondria which fill the entire cytoplasm, except for a sharply delimited crescent-shaped clear area surrounding the pit. In some of the pitted cells this clear region extends as a broad path to a point just above the nucleus (Fig. 4). In the fresh water adapted cell, the mitochondria are more loosely packed.

The osmiophilia varies in amount in both sea and fresh water cells. There is no evidence that it assumes a characteristic form in either adaptation. The Golgi element, which is present in cells adapted to either salinity, usually consists of large granules or bent rods of osmiophilic material arranged in a band across the cell in the supranuclear position (Fig. 6). It is often obscured by varying amounts of a fine granular osmiophilia distributed throughout the cell. The granular osmiophilia, when present, is always evenly distributed throughout the cell, even including the clear areas observed about the pits in Regaud preparations.

Transitional adaptation. Changes in the chloride cell as it adapts to a new salinity are not abrupt. A gradually increasing number of pits is seen in the cells of eels transferred from fresh water to sea water. The cells of animals killed after 2 hours in sea water still have the appearance of those in the fresh water adapted animal, only a few pitted cells being present. The cells of animals stimulated for 6 hours are the first to show a greater abundance of pits. At the end of 15 hours, the filaments appear to be sea water adapted. The chloride cells become more narrow and the mitochondria more densely packed. In the group stimulated for 24 hours, the number of pitted cells does not appear to have increased over that of the 15 hour animals.

The animals transferred to fresh water after 3 days in sea water similarly exhibit gradual changes as they adapt to a lesser salinity. The 2 hours group has the appearance of the sea water adapted animal, but in the 6 hour, and particularly in the 15 and 24 hour animals, there is an evident diminution in the number of pitted cells. A clear area is visible at the free surface in some of the fresh water adapted cells and the mitochondria are less densely packed than in the sea water phase. The animals in the 15 and 24 hour series appear adapted to fresh water.

Discussion

The cytological picture seen in the sea water adapted eel is similar to that found in marine *Fundulus heteroclitus* by Copeland (1948, 1950). Both show a definite structure in the distal end of the sea water adapted cell, the round "excretory vesicle" in Fundulus and the narrow pit in Anguilla. The distribution of mitochondria is similar in the two animals. The amount of osmiophilic material present varies among the cells adapted to a particular salinity and no one picture is apparently typical of the external salinity to which the cell is exposed.

There is not complete agreement in the morphological change during adaptation. When Fundulus adapts to fresh water, the excretory vesicle disappears. This is not paralleled by a complete disappearance of the pit in the chloride cells of Anguilla; instead, an occasional pit persists.

Salt water adaptation. Observations by Keys (1933) on eels and by Smith (1930) on other marine teleosts show that these fish swallow sea water to provide a source of water for the hypotonic urine. The consequent excess of salt in sea water adapted animals is excreted mainly through the gills, and the pits in the chloride cells reflect the operation of an osmotic regulatory process.

The changes in the chloride cell, in animals adapting to sea water, roughly parallel the changes in serum chloride concentration measured by Keys (1933). He notes that movement of water, most of which probably leaves the body by way of the gills, begins as soon as the animal is transferred from fresh to salt water and that chloride is moved across the gills only after a considerable rise in internal concentration occurs as a result of the initial movement of water alone. The present work tends to bear this out. The cells of the 2 hour series appear like those of the fresh water adapted animals and not until the 6 hour series is the quantity of pits typical of the sea water condition begun to be found. It would appear that between the second and sixth hour a threshold had been reached and active excretion begun. Eels of the 15 hour series appear to be completely adapted cytologically, although Keys' experiments show the process is not physiologically complete until about 48 hours.

Fresh water adaptation. Keys' experiments with the eel adapting to fresh water show that water enters the body and that the serum chloride concentration is lowered. He also found, when he used the heart-gill perfusion system, that the gills stop active salt excretion. Cytologically, we find a measurable decrease in the number of chloride cells with pits in animals adapting to fresh water. They never disappear completely, even in the animals which are considered to be completely adapted (3 to 5 days in fresh water). There are no quantitative measurements available to tell whether or not the eel in fresh water continues to transport a small amount of chloride across its gills. The cytological evidence would indicate that possibility.

It is also possible that the presence of a few pits may not be the normal cytological picture, in view of the conditions under which the animals were kept. Grafflin (1931) reported the marked diuresis in marine teleosts kept in live cars, and Meyer (1948) has noted the failure of goldfish to absorb salt from dilute solutions for some hours after the fish were handled. The eels used in this study may have suffered slight injuries to the skin in the traps or in the somewhat crowded aquaria used for adaptation. In view of the importance which Grafflin and Meyer have shown the immediate environment to have on the physiological state of the animal, a true picture of the chloride cell may not be possible unless bits of gill from animals taken directly from their natural habitat are fixed and observed.

Nevertheless, a distinct difference in the appearance of the chloride cells of eels adapted to fresh or salt water exists. On the basis of the physiological results obtained by Smith and Keys and the cytological picture in Fundulus and Anguilla, the following hypothesis is made. The presence of pits is evidence of chloride excretion in relatively large amounts per cell. Their absence characterizes fresh water adaptation and possible chloride absorption in relatively small amounts per cell. The presence of a few pits in the fresh water Anguilla may indicate a failure to completely adapt to fresh water due to the environmental conditions of the experiment (Grafflin, 1931; Meyer, 1948).

The cytological interpretation of Krogh's physiological observation that Anguilla does not absorb salts in fresh water is not certain. The obvious interpretation of Krogh's results is that salts are not moved at all under fresh water condition. That could be modified by the above hypothesis to the following: Due to different threshold sensitivities of the cells to blood concentrations, a few fail to change to the absorptive condition. The many cells in the absorbing phase are negated by the few cells in the excreting phase, the result being very little net gain or loss.

In conclusion, I wish to thank Dr. D. E. Copeland for suggesting this problem and for his helpful advice and criticism throughout the investigation.

SUMMARY

1. The shape of the chloride cells in the gill of *Anguilla rostrata* varies with their position in the filament. The cells along the free edge have a thin, spindle shape, while those at the bases of the respiratory platelets are more round.

2. Eels adapted to sea water exhibit narrow pits at the free surface of the chloride cells that are located along the afferent artery.

3. The presence of many pits is characteristic of sea water adaptation, but animals adapted to fresh water also possess an occasional pitted cell.

4. The varying number of pits in fresh and sea water adapted animals is correlated with the findings from previous physiological studies of osmotic adaptation.

LITERATURE CITED

COPELAND, D. E., 1948. The cytological basis of chloride transfer in the gills of *Fundulus heteroclitus*. *J. Morph.*, **82**: 201–227.

COPELAND, D. E., 1950. The adaptive behavior of the chloride cell in the gill of *Fundulus heteroclitus*. *J. Morph.* (in press).

GRAFFLIN, A. L., 1931. Urine flow and diuresis in marine teleosts. *Am. J. Physiol.*, **97**: 602–610.

KEYS, A., 1931. Chloride and water secretion and absorption by the gills of the eel. *Zeit. vergl. Physiol.*, **15**: 364–388.

KEYS, A., 1933. The mechanism of adaptation to varying salinity in the common eel and the general problem of osmotic regulation in fishes. *Proc. Roy. Soc. London, Ser. B.*, **112**: 184–199.

KEYS, A. AND E. N. WILLMER, 1932. "Chloride secreting cells" in the gills of fishes, with special reference to the common eel. *J. Physiol.*, **76**: 368–378.

KROGH, A., 1937. Osmotic regulation in fresh water fishes by active absorption of chloride ions. *Zeit. vergl. Physiol.,* **24**: 656–666.

LUDFORD, R. J., 1926. Further modifications of the osmic acid methods in cytological techniques. *J. Roy. Micro. Soc.,* **46**: 107–109.

MEYER, D. K., 1948. Physiological adjustments in chloride balance of the gold fish. *Science,* **108**: 305–307.

PETTENGILL, O. AND D. E. COPELAND, 1948. Alkaline phosphatase activity in the chloride cell of *Fundulus heteroclitus* and its relation to osmotic work. *J. Exp. Zool.,* **108**: 235–242.

SMITH, H., 1930. The absorption and excretion of water and salts by marine teleosts. *Am. J. Physiol.,* **93**: 480–505.

A COMPARISON OF NUTRITIVE VALUES AND TASTE THRESHOLDS OF CARBOHYDRATES FOR THE BLOWFLY

C. C. HASSETT, V. G. DETHIER, AND J. GANS

The Medical Division, Army Chemical Center, Maryland, and the Department of Biology, The Johns Hopkins University

The question has often been raised as to the correspondence between the nutritive value of various substances and their acceptability by animals. Several species of vertebrates and invertebrates have been investigated in this respect. In the rat, for example, Richter (1943) has demonstrated a definite relation between the nutritive value of purified components of various diets and their acceptability. In insects the relation is less definitely established. Only two species have been investigated thoroughly. The results of Vogel's (1931) and von Frisch's (1934) work with the honeybee and Haslinger's (1935) with the fleshfly *Calliphora erythrocephala* showed that substances which are without nutritive value are tasteless but that those with nutritive value may be tasted or not.

Because of the few species of insects tested and the small number of compounds available at that time the status of the question in this class of animals remains in some doubt. Hence, before data from the aforementioned experiments alone become established as a general rule they should be augmented by information derived from studies of additional species and a greater number of compounds.

Few insects are favorable for such combined tests, usually for lack of a suitable technique for measuring sensory responses. Consequently, the nutritional phase of the problem has received the greater share of attention. Bertholf (1927) and Phillips (1927) studied the nutritive value of different compounds for the honeybee; Haslinger (1935) and Fraenkel (1940), for *Calliphora;* Baker *et al.* (1944), for *Anastrepha ludens;* and Hassett (1948), for *Drosophila melanogaster.*

The development of a reliable method for studying the sensory responses of tarsal chemoreceptors by Minnich (1921) and a method for analyzing the data obtained (cf. Bliss, 1938; Dethier and Chadwick, 1948) has opened a way for an extension of the work, begun by Haslinger, on muscoid flies.

The investigation to be described here was divided into two parts: one designed to ascertain the nutritive value of carbohydrates and related substances for the blowfly *Phormia regina* Meigen; the other, an examination of the stimulating effectiveness of these substances for the tarsal chemoreceptors. It is hoped that such an experiment involving large numbers of flies for both phases of the subject will contribute materially toward the solution of the problem.

METHODS

The flies used in this work were reared according to the method of Hill, Bell, and Chadwick (1947). For survival tests, pupae were placed in clean jars, and on the day of emergence lots of 100 flies were transferred to 15 × 15 × 15 cm. screen cages. Males and females in approximately equal numbers were used. The cage front consisted of a sliding glass panel which rested on another glass plate used as a liner for the cage bottom. This bottom plate projected about 2 cm. under the sliding door. On the step thus formed was inverted a vial of the solution to be tested. A strip of filter paper conducted the solution from the vial into the cage and formed a convenient feeding surface. All substances tested were offered in M/10 solution; the vials and paper were changed daily. Dead flies were removed and counted each day by carefully withdrawing the bottom plate on which they lay. During the experiment the flies were kept in a room at 25° C. and 70 per cent R.H.

TABLE I

The survival of blowflies on various compounds, given as summations of daily survival percentages (A), and as days required for 50 per cent mortality (B). M/10 solutions. Each test represents 100 flies

	A	B		A	B
Controls			Disaccharides		
Water, 70% R.H.	212	2½	Maltose	2259	23
Nothing, 70% R.H.	174	2	Sucrose	1573	16
Water, 30% R.H.	120	2	Trehalose	1287	15
Nothing, 30% R.H.	72	1	Melibiose	874	6
			Lactose	339	3½
Pentoses			Cellobiose	264	3½
D-Xylose	367	4½	Trisaccharides		
D-Ribose	343	4			
L-Rhamnose	302	3½	Melezitose	1760	20
D-Lyxose	287	3½	Raffinose	1295	13
L-Arabinose	264	3½			
L-Fucose	227	2½	Alcohols		
D-Arabinose	107	2			
			Sorbitol*	1332	15
Hexoses			Glycerol*	363	4½
			Inositol	299	3½
D Mannose	1914	18	Dulcitol†	243	3
D-Glucose	1636	16½	m-Erythritol‡	247	3
D-Galactose	1636	17	penta-Erythritol‡	204	2½
D-Fructose	1556	14			
L-Sorbose	280	3½	alpha-Methylglucoside§	800	9

* U.S.P., Colgate & Co.

† Eimer and Amend.

‡ Supplied through courtesy of Dept. Agr. Chem., The Ohio State Univ.

§ Eastman Kodak Co.

Data on survival have been calculated as the number of days from emergence to the time at which 50 per cent remained alive, and also as a score based on the summation of the daily percentage of survival.

The technique employed in testing the stimulating effectiveness of compounds applied to the tarsi of flies has been described fully by Minnich (1929) and Dethier and Chadwick (1947). It makes use of a convenient reflex, the proboscis response. When receptors located on the tarsi are stimulated by solutions of certain substances, among them being sucrose, glucose, and fructose, the insect responds by extending the retractible proboscis and, when permitted, by drinking. The lowest concentration of a sugar which will elicit this response is termed the acceptance threshold.

TABLE II

The response of Phormia to sugars and polyhydric alcohols

Sugar	Log molar conc. accepted by 50% ±2.575 S.E.	a ± S.E.*	b ± S.E.*	$\bar{x}$*	No. of flies tested
Disaccharides					
Sucrose	−2.009 ± 0.319	5.033 ± 0.130	1.045 ± 0.168	−1.978	180
D-Maltose	−2.362 ± 0.631	5.324 ± 0.158	0.750 ± 0.221	−1.931	80
D-Trehalose	−0.875 ± 0.324	4.872 ± 0.148	1.174 ± 0.195	−0.984	120
Cellobiose	20% of the flies accepted at 1.5 molar				60
Lactose	refused in saturated solution by all flies				80
Melibiose	refused in saturated solution by all flies				
Trisaccharides					
Melezitose	−1.196 ± 0.258	4.891 ± 0.147	1.460 ± 0.187	−1.270	120
Raffinose	−0.698 ± 0.131	5.180 ± 0.140	2.791 ± 0.330	−0.633	120
Monosaccharides					
D-Fructose	−2.238 ± 0.131	4.977 ± 0.164	3.252 ± 0.428	−2.245	100
L-Fucose	−1.060 ± 0.245	5.004 ± 0.164	1.731 ± 0.249	−1.058	120
D-Glucose	−0.880 ± 0.227	5.153 ± 0.158	1.782 ± 0.270	−0.794	140
L-Sorbose	−0.854 ± 0.165	4.963 ± 0.164	2.552 ± 0.338	−0.868	100
D-Arabinose	−0.843 ± 0.095	5.429 ± 0.216	3.297 ± 0.675	−0.714	200
L-Xylose	−0.472 ± 0.219	5.120 ± 0.160	1.874 ± 0.329	−0.408	120
D Xylose	−0.357 ± 0.234	5.077 ± 0.148	1.630 ± 0.232	−0.310	120
D-Galactose	−0.302 ± 0.170	4.967 ± 0.148	2.277 ± 0.295	−0.316	120
L-Arabinose	−0.271 ± 0.093	4.832 ± 0.083	2.344 ± 0.202	−0.343	360
D-Mannose	50% of the flies accepted at 7.59 molar				
D-Ribose	0.954 ± 0.752	4.012 ± 0.187	1.530 ± 0.635	0.312	80
D-Lyxose	1.626 ± 1.306	3.708 ± 0.192	1.194 ± 0.513	0.541	100
L-Rhamnose	refused in saturated solution by all flies				
2-Desoxyribose	refused in saturated solution by all flies				
α-Methyl glucoside	−1.159 ± 0.080	5.007 ± 0.114	3.674 ± 0.322	−1.157	240
Inositol	−0.7125 ± 0.160	5.027 ± 0.125	2.012 ± 0.241	−0.699	140

* The 3rd, 4th, and 5th columns of the table give the calculated values for a, b, and $\bar{x}$ in the equation $Y = a + b(X - \bar{x})$, which is the regression of per cent flies accepting, Y, expressed as probits, on log concentration, X. S.E. = standard error.

The sugars and other compounds used in these experiments, with the exception of those noted in Table I, were obtained from Pfanstiehl Chemical Company and were C.P. reagents.

RESULTS

Under the conditions specified for the survival tests, control flies given only water live 2.5 days on the average. If not given water they die somewhat sooner. In a dry (30 per cent R.H.) atmosphere, however, flies survive two days when water is available and only one day when it is not. A humid atmosphere seems to be more important than available drinking water. These results and those from the feeding tests are shown in Table I.

Since any substance which increases survival materially beyond that of the control flies can be assumed to have some nutritive value, it is evident that all the pentoses tested except fucose are utilized to some extent, with xylose and ribose as the best of a rather poor group. The hexoses are almost uniformly good, sorbose being the single exception. Of the disaccharides, maltose, sucrose, and trehalose are good, the first exceptionally so. Melibiose is intermediate; lactose and cellobiose are poorly utilized. The trisaccharides melezitose and raffinose, and one alcohol, sorbitol, complete the list of well utilized compounds. Alpha-methylglucoside is of moderate value, but the polyhydric alcohols are poor with the exception just noted.

Data showing the acceptance thresholds for *Phormia* are given in Table II. When due consideration is given to the variance of the medians, it is clear that many of the apparent differences in the effectiveness of the various sugars disappear. In general, with this stock of *Phormia* and with these testing methods, a difference in median values to be significant at the 1 per cent level must represent not less than 1½ doubling concentration steps. Under these conditions the effectiveness of the sugars tabulated above is: *disaccharides*—sucrose = maltose > trehalose > cellobiose > lactose; *monosaccharides*—fructose > fucose = glucose = D-arabinose = sorbose > D-xylose = L-xylose = galactose = L-arabinose > mannose = ribose > lyxose.

Discussion

As regards the utilization of various compounds by *Phormia,* a careful examination of the data and a comparison of them with earlier work discloses no significant differences. Minor differences were found such as the utilization by *Phormia* of rhamnose and cellobiose, which other species do not use; but the nutritional value of these substances is so slight that this fly can hardly be said to vary from *Calliphora* or *Drosophila* in its ability to utilize the various carbohydrates. The presence of both an alpha-glucosidase and an alpha-galactosidase is clearly demonstrated. In addition, the tests with lactose and cellobiose indicate a rather low level of activity by a beta-glucosidase and a beta-galactosidase.

Taste thresholds for a large series of carbohydrates have been measured for only seven species of insects. A reëvaluation of the data of Weis (1930) for *Pyrameis atalanta,* of von Frisch (1934) for the honeybee, Haslinger (1935) for *Calliphora,* and Schmidt (1938) for three species of ants shows remarkable similarities to those for *Phormia* when all species are compared (Table III).

In considering Haslinger's results with *Calliphora* it must be remembered that his tests were run with comparatively small numbers of flies, from 20 to 48, and that insufficient data are presented to permit calculation of the variance. It is unlikely, however, that the values obtained with these numbers are any more reliable than those obtained with *Phormia* where from 60 to 360 flies were tested with each sugar. If we may apply the same analysis to Haslinger's data that we have to our own, the following is the order of stimulating effectiveness for the sugars for the legs: *disaccharides*—sucrose = maltose > trehalose = cellobiose > lactose; *monosaccharides*—fructose > fucose > glucose = xylose = galactose = arabinose = mannose; for the mouth *disaccharides*—sucrose = maltose > trehalose = cellobiose >

TABLE III

A comparison of acceptance thresholds (molar concentration) of sugars and polyhydric alcohols for different insects

Compounds	*Phormia* (tarsi) median threshold	*Calliphora* (tarsi mouthparts) starvation threshold (data from Haslinger)		*Apis* (mouthparts) data from v. Frisch	*Pyrameis* (tarsi) medians calculated from Weis' data	*Lasius niger*	*Myrmica rubra*	*Myrmica rubida*
						(mouthparts) minimum threshold (data from Schmidt)		
Sucrose	0.0098	0.0006	0.0035	0.0625–0.125	0.02	0.005	0.0066	0.0025–0.00125
D-Maltose	0.0043	0.00125	0.002	0.0125	0.45	0.01	0.01	0.01
D-Trehalose	0.133	0.14	0.02	0.25	>1.0	—	—	—
Lactose	*	*	0.1	*	*	*	*	*
Cellobiose	5.01	0.05	0.03	*	>1.0	*	—	*
Melibiose	*	*	*	*	—	—	*	*
Melezitose	0.064	0.07	0.01	0.125–0.25	0.93	0.01	0.005	0.0156
Raffinose	0.20	0.10	0.007	*	0.45	0.0025	0.0025	0.01
D-Fructose	0.0058	0.0033	0.004	0.25	0.15	0.0156	0.02	0.03125
L-Fucose	0.087	0.02	0.01	1.0	0.03	—	—	—
D-Glucose	0.132	0.125	0.04	0.25	0.20	0.125	0.02	0.03125
L-Sorbose	0.140	—	—	—	—	—	—	—
D-Arabinose	0.144	—	—	*	—	—	—	—
L-Xylose	0.337	—	—	—	—	—	—	—
D-Xylose	0.440	0.2	0.14	*	>1.0	—	—	*
D-Galactose	0.50	0.14	0.09	2.0	>1.0	*	0.5	*
L-Arabinose	0.536	0.1	0.08	*	0.85	*	*	*
D-Mannose	7.59	0.2	0.1	*	*	*	0.25	*
D-Ribose	8.99	—	—	—	—	—	—	—
D-Lyxose	42.27	—	—	—	—	—	—	—
2-Desoxyribose	*	—	—	—	—	—	—	—
L-Rhamnose	*	*	1.0	*	—	*	—	*
α-Methyl glucoside	0.069	0.01	0.03	0.50	0.20	0.125	0.02	0.125
Inositol	0.194	0.03	0.03	ca 0.25	*	—	—	—
Glycerol	*	—	—	—	—	—	—	—
Sorbitol	*	0.03	0.08	*	—	0.25	0.0625	*
Dulcitol	*	0.03	0.08	*	>1.0	*	*	*
Mannitol	*	0.1	0.14	*	>1.0	*	1.0	*
M-erythritol	*	—	—	*	*	*	*	*
Penta-erythritol	*	—	—	—	—	—	—	—

* Non-stimulating at all concentrations. —Not available for testing.

lactose; *monosaccharides* — fructose ≧ fucose > glucose ≧ arabinose ≐ xylose = galactose = mannose. A similar treatment of Weis' data gives the following order: *disaccharides*—sucrose > maltose > trehalose ? cellobiose > lactose; *monosaccharides*—fucose > fructose > glucose > L-arabinose > xylose ? galactose > mannose. Schmidt's data are in the order: *disaccharides*—sucrose ≧ maltose > cellobiose; *monosaccharides*—fructose ≧ glucose > galactose. Von Frisch worked with considerably larger numbers of bees in his field experiments. For disaccharides the order of effectiveness is: sucrose = maltose > trehalose; for *monosaccharides*, fructose = glucose > fucose > galactose. All other sugars which were tested with bees failed to stimulate in saturated solution.

Considered thus, the outstanding features of the eight sets of threshold data are the similarities rather than the differences (Table III). The three outstanding discrepancies are the value of cellobiose, which is considerably stimulating to *Calliphora;* the value of fucose, which to the bee is less stimulating than glucose and to *Pyrameis* is more stimulating than fructose; the value of raffinose, which is very low for the mouthparts of *Calliphora* and for ants. The fact is inescapable, however, that the most stimulating disaccharides are those which contain glucose or fructose units and can be hydrolized by alpha glucosidases, and the most stimulating monosaccharides are fructose, glucose, and fucose. The polyhydric alcohols are, in general, ineffective, but inositol is appreciably stimulating to *Phormia* and *Calliphora.* Further comparisons are impossible because of the lack of uniformity of testing methods employed by different authors. It should be noted that each reports different criteria of stimulating effectiveness.

TABLE IV

A comparison of the nutritive value of carbohydrates and related compounds with the acceptance thresholds for Phormia

Compound	Comparative nutritional value	Molar concentration at threshold
D-Maltose	++	0.0043
D-Fructose	++	0.0058
Sucrose	++	0.0098
Melezitose	++	0.064
α-Methylglucoside	++	0.069
L-Fucose	−	0.087
D-Glucose	++	0.132
L-Sorbose	−	0.140
D-Arabinose	−	0.144
Inositol	+	0.194
Raffinose	++	0.200
L-Xylose	+	0.337
D-Xylose	+	0.440
D-Galactose	++	0.500
L-Arabinose	+	0.536
Cellobiose	+	5.01
D-Mannose	++	7.59
D-Ribose	+	8.99
D-Lyxose	+	42.27
Melibiose	++	*
Sorbitol	++	*
Glycerol	+	*
Dulcitol	+	*
Meso-Erythritol	+	*
Penta-Erythritol	−	*

The data in hand from both phases of this study indicate that it is not possible to correlate the taste of a substance and its nutritive value, as Vogel was able to do to some extent. Her data for the honeybee show that all sweet substances are utilized while both nutritious and non-nutritious substances are included in the tasteless group. The present study reveals, on the contrary, that all gradations of taste thresholds occur for nutritious and non-nutritious substances. If these materials be arranged in order of their threshold values (Table IV), most of the substances which are well utilized are found among those with low threshold values, but mannose, at the top nutritionally, has a threshold far above the level it might be expected to occupy; conversely, fucose, with zero value as food, is very stimulating.

On the basis of all studies to date it would appear that with some insects at least there is no fixed correlation between the nutritive value of substances and their acceptability.

SUMMARY

Thirty sugars and related compounds have been tested on the blowfly *Phormia regina* Meigen to determine their nutritive value for this species and their effectiveness as stimuli for the tarsal receptors.

Survival tests indicated that all pentoses except fucose are utilized to some extent. Xylose and ribose are the best. The hexoses are almost uniformly good. Sorbose is the exception. Maltose is the most effectively utilized disaccharide. Sucrose and trehalose are good; melibiose, intermediate; lactose and cellobiose, poor. The trisaccharides melezitose and raffinose and the alcohol sorbitol are effective. In general the polyhydric alcohols are poor.

The stimulating effectiveness of the sugars when applied to the tarsi is as follows: *disaccharides*—sucrose = maltose > trehalose > cellobiose > lactose; *monosaccharides*—fructose > fucose = glucose = D-arabinose = sorbose > D-xylose = L-xylose = galactose = L-arabinose > mannose = ribose > lyxose.

There is no good correlation between the nutritive value of these compounds and their acceptability.

LITERATURE CITED

BAKER, A. C., W. E. STONE, C. C. PLUMMER AND M. MCPHAIL, 1944. A review of studies on the Mexican fruitfly and related Mexican species. *U. S. Dept. Agr., Misc. Pub. 531,* 155 p.

BERTHOLF, L. M., 1927. The utilization of carbohydrates as food by honey-bee larvae. *J. Ag. Res.,* **35**: 429.

BLISS, C. I., 1938. The determination of the dosage-mortality curve from small numbers. *Quart. J. Pharm. and Pharmacol.,* **11**: 192.

DETHIER, V. G. AND L. E. CHADWICK, 1947. Rejection thresholds of the blowfly for a series of aliphatic alcohols. *J. Gen. Physiol.,* **30**: 247.

DETHIER, V. G. AND L. E. CHADWICK, 1948. The stimulating effect of glycols and their polymers on the tarsal receptors of blowflies. *J. Gen. Physiol.,* **32**: 139.

FRAENKEL, G., 1940. Utilization and digestion of carbohydrates by the adult blowfly. *J. Exp. Biol.,* **17**: 18.

VON FRISCH, K., 1934. Über den Geschmackssinn der Biene. *Zeit. vergl. Physiol.,* **21**: 1.

HASLINGER, F., 1935. Über den Geschmacksinn von Calliphora erythrocephala Meigen und über die Verwertung von Zuckern und Zuckeralkoholen durch diese Fliege. *Zeit. vergl. Physiol.,* **22**: 614.

HASSETT, C. C., 1948. The utilization of sugars and other substances by Drosophila. *Biol. Bull.,* **95**: 114.

HILL, D., V. A. BELL, AND L. E. CHADWICK, 1947. Rearing of the blowfly, *Phormia regina* Meigen, on a sterile synthetic diet. *Ann. Ent. Soc. Amer.,* **40**: 213.

MINNICH, D. E., 1921. An experimental study of the tarsal chemoreceptors of two nymphalid butterflies. *J. Exp. Zool.,* **33**: 173.

Minnich, D. E., 1929. The chemical sensitivity of the legs of the blowfly *Calliphora vomitoria* Linn., to various sugars. *Zeit. vergl. Physiol.,* **11**: 1.

Phillips, E. F., 1927. The utilization of carbohydrates by honeybees. *J. Ag. Res.,* **35**: 385.

Richter, C. P., 1943. The self-selection of diets. *Essays in Biology, Univ. Calif. Press.* pp. 501–506.

Schmidt, A., 1938. Geschmacksphysiologische Untersuchungen an Ameisen. *Zeit. vergl. Physiol.,* **25**: 351.

Vogel, B., 1931. Über die Beziehungen zwischen Süssgeschmack und Nährwert von Zuckern und Zuckeralkoholen bei der honigbiene. *Zeit. vergl. Physiol.,* **14**: 273.

Weis, I., 1930. Versuche über die Geschmacksrezeption durch die Tarsen des Admirals, *Pyrameis atalanta* I. *Zeit. vergl. Physiol.,* **12**: 206.

STUDIES IN THE REGULATION OF BLOOD-SUGAR CONCENTRATION IN CRUSTACEANS. II. EXPERIMENTAL HYPERGLYCEMIA AND THE REGULATORY MECHANISMS[1]

L. H. KLEINHOLZ, *with the assistance of* V. J. HAVEL *and* R. REICHART

Reed College, Portland, Oregon, and the Marine Biological Laboratory, Woods Hole, Mass.

In the first paper of this series (Kleinholz and Little, 1949), the literature of hyperglycemia in crustaceans was reviewed and some experimental conditions effecting increase in total reducing substances of crustacean blood were examined. This increase in total reducing substances of the blood was shown to be in the fermentable component, and therefore probably represented a true hyperglycemia. The wide range of these glycemic values, sometimes even in the same species studied by different investigators, soon led to recognition of the need of maintaining animals under known laboratory conditions, and to the employment of standard analytical methods. With these precautions, experimental modification of the glycemic level in crustacea became more reliable. The studies of Hemmingsen (1924), later extended by Lindblad (1931), on the rate of disappearance of injected glucose from the blood of Astacus, indicated the possible presence of a hypoglycemic regulatory mechanism. Little attempt has been made to localize this mechanism anatomically.

A group of subsequent studies by various investigators, probably influenced by the known glycemic effects of adrenaline and insulin among vertebrates, reported results of injecting these substances into crustaceans. Some of the results were contradictory and for a time discussion centered not so much about the interpretation of the reported experimental results as about the specificity of the particular pharmacological agent in producing its effect. Thus, Roche and Dumazert (1935) stated that neither insulin nor adrenaline had any effect on the blood-sugar concentration of *Cancer pagurus*. Medvedeva (1936) found that injection of adrenaline in Potamobius (syn. Astacus) caused hyperglycemia, but that injection of insulin had no definite effect. Kalmus and Waldes (1936), questioning the specificity of the adrenaline-induced hyperglycemia, observed that not only adrenaline and insulin, but also such non-specific substances as hydroquinone and sodium chloride resulted in marked increases in blood-sugar concentration when injected into crayfish. Florkin and Duchateau (1939) attempted to resolve some of these contradictions by more carefully controlled procedures, and reported that insulin had no effect while adrenaline produced hyperglycemia in crayfish, thus confirming the earlier observations of Medvedeva.

These observations, questionable as may have been the interpretation of the specificity of the pharmacologcial effect, indicated the possible existence of a hyperglycemic mechanism (as Hemmingsen's studies had indicated a *hypoglycemic*

---

[1] This study was aided by grants from the Penrose Fund of the American Philosophical Society, and from the Permanent Science Fund of the American Academy of Arts and Sciences.

mechanism). Abramowitz, Hisaw and Papandrea (1944) demonstrated that a definite anatomical structure might be involved in this hyperglycemic mechanism. These investigators found that injection of aqueous extracts of crustacean eyestalks into *Callinectes sapidus* brought about a marked increase in blood-glucose concentration; more specific localization showed the source of this hyperglycemic or diabetogenic hormone to be associated with the sinus gland within the eyestalk, a structure that has already been demonstrated to participate in a number of endocrine processes in crustacea (Kleinholz, 1942; Brown, 1948).

Our own interest in investigating the nature of this hyperglycemic mechanism was stimulated by indications in the literature, not further explored, of hyperglycemic responses in crustaceans similar to the excitement hyperglycemia reported in mammals. Stott (1932) had reported a large increase in blood-sugar concentration of several crustaceans after asphyxiation, obtained by keeping them for 10 hours in tightly-covered containers filled with sea water. Roche and Dumazert (1935) confirmed this observation on *Cancer pagurus;* they found that removing animals from sea water and keeping them in air for 30 to 60 minutes resulted in marked hyperglycemia. Both studies reported this increase in glycemic level as a direct observation without investigating in further detail the mechanism of this response. In the first paper of this series (Kleinholz and Little, 1949) similar results were obtained with Libinia, and a possible mechanism for this hyperglycemia was indicated by the fact that animals from which both eyestalks had been removed no longer showed great increases in blood-sugar after asphyxia.

The next logical and traditional development, after injection of extracts, in proving hormonal participation of sinus gland in blood-sugar regulation, would be actual demonstration of the mediation of the sinus gland in experimentally-produced hyperglycemia. Removal of sinus glands by ablation of eyestalks, as was done with Libinia, is not sufficiently critical to be considered conclusive proof of such mediation for reasons which are explained below in the next section on methods. Surgical removal of the sinus gland from the eyestalk of Libinia is difficult because of the small size of the stalk and its lesser accessibiltiy than in other crustaceans. More exact studies in which sinus glands were removed with little damage to the rest of the eyestalk were postponed until the time when surgically more convenient animals like Astacus and Callinectes could be used.

The present report describes experiments which prove conclusively the mediation of the sinus gland in cases of physiologically-induced hyperglycemia, and which investigate the resemblance between experimental hyperglycemia in crustaceans and the excitement hyperglycemia of higher vertebrates.

MATERIALS AND METHODS

The animals used in this study were the common Oregon crayfish, *Astacus trowbridgii,* and the blue crab of the Atlantic Coast, *Callinectes sapidus.* Crayfish were maintained in the laboratory in tanks through which tap water circulated, a depth of about two inches of water being maintained. Each of these tanks was covered to provide shaded areas, while the tanks themselves were located in an unheated, well-ventilated room. A supply of Callinectes was kept in a large outdoor concrete tank supplied with running sea water.

Stock crayfish were fed approximately once a week on freshly-killed frogs, clams, or bits of beef. The storage tank in which Callinectes were kept had an abundance of food material in the form of freshly-killed fish and discarded crabs which the living crabs readily scavenged. The individuals constituting some of the early experimental groups were isolated and starved for 3 to 5 days before undertaking any experimental procedure. In later groups individuals were isolated for no more than a day because the observations reported here are concerned chiefly with relative changes in a period of a few hours rather than with absolute changes in the concentration of blood-sugar.

At least one day before taking the first blood sample the carapace of each crayfish was punctured with a dissecting needle in the mid-dorsal line directly over the pericardial cavity, and the hole then plugged with a bit of modelling clay. In taking blood samples from Astacus this plug was removed and about 0.15 ml. of blood taken by means of a 20-gauge hypodermic needle fitted to a graduated 1 ml. tuberculin syringe. The opening into the pericardial cavity was re-sealed with modelling clay, and the animal returned either to water or to its particular experimental environment. In Callinectes blood samples were taken by puncturing the arthrodial membrane at the base of one of the walking legs with the needle of the hypodermic syringe. An exact 0.10 ml. blood sample was transferred from the syringe to a calibrated serological pipette. The blood was then blown into a tube containing acid cadmium sulfate and rinsed half a dozen times with this deproteinizing mixture. Blood samples, both under control and experimental conditions (except where asphyxia of Astacus was attempted by prolonged maintenance in air), were always taken in the fore-noon to avoid the possibility of diurnal and nocturnal fluctuations in the concentration of blood-sugar. Male individuals only were used in these studies, the crayfish ranging from about 80 to 100 grams and the crabs from about 120 to 150 grams in weight.

Groups of six animals were used at a time. In all cases blood samples were taken from the individuals of a group before a particular experimental treatment and then a second time immediately after such treatment. In this way each animal served as its own control to the experimental condition used. Both the control and the experimental blood samples received comparable handling, being carried through the analytical procedure simultaneously. The method of Miller and Van Slyke (1936) was used in determining the blood-sugar concentration of the samples. Three blanks were prepared with each set of control and experimental blood samples. These blanks required from 0.13 to 0.28 ml. of the dilute ceric sulfate to attain the same end-point reached in the titration of the blood samples. The average volume of this reagent required for 120 such blanks was 0.184 ml.; this compares with an average of 0.195 ml. for 58 similar blanks in the preceding study of this series (Kleinholz and Little, 1949). No distinction was attempted between fermentable and non-fermentable components of the blood, so that the values reported in this study represent the total reducing substances expressed as glucose equivalents.

Effective asphyxia which had been obtained with Libinia by removing the animal from water to an air environment was similarly accomplished with Callinectes. This method, however, was not successful with Astacus. Asphyxia was therefore induced by immersing these animals for stated periods in de-aerated water. A heavy-walled flask containing 2 liters of water was de-aerated by connecting the flask to a vacuum pump and maintaining the reduced pressure for 15 minutes after vigorous bubbling had commenced. At the end of this time the vacuum was broken and the water decanted into screw-top jars of about 500 ml. capacity. Each jar was filled to the brim and a crayfish lowered into it, the displaced water overflowing the jar; the screw-top, fitted with a rubber gasket, was applied to exclude air bubbles and tightened. At the end of the period of asphyxia the animal was removed from the jar and the second blood sample taken. Crayfish were usually limp after this asphyxia, but recovered rapidly after being returned to their tanks.

The effect of chloroform anesthesia on concentration of blood-sugar was studied by immersing crayfish for various periods of time in aqueous solutions of chloroform. The solutions were prepared by vigorously shaking small measured volumes of chloroform with 500 to 1000 ml. of water and then diluting with additional volumes of water to give the desired concentrations. Enough solution was prepared and poured into a small tank so that the surface was about one-half inch above the dorsal carapace of the immersed individual. After the periods of immersion the animals were removed and blood samples taken for the second time before the crayfish were returned to their stock tanks for recovery.

Study of the effect of adrenaline on the concentration of blood-sugar was made by injecting dilutions (in distilled water for Astacus and in 0.7% saline for Callinectes) of Parke Davis & Company "Adrenalin (1 : 1000)" into the blood sinus at the base of a walking leg. These dilutions and the volume injected (0.1 to 0.2 ml.) were arranged to give a series ranging from $150\,\gamma$ to $0.1\,\gamma$. Comparable volumes of distilled water, of 0.7% saline, and of saturated aqueous chloretone were used as control injections. The dilutions were prepared on each occasion of use from 1 ml. ampoules. In this group of experiments each animal served as its own control, the procedure being to take the first set of blood samples before injecting the desired solution and then, 90 minutes after the injection, to take the second blood sample.

The role of the sinus gland in the hyperglycemic responses obtained by the various procedures described above was determined by repeating these procedures on animals from which the sinus glands had been removed. In many early studies removal of the sinus gland was effected by simple ablation of both eyestalks, this procedure being frequently dictated by the difficulties of surgical operation on very small eyestalks. Such investigations could be criticized on the basis that ablation of entire eyestalks removed simultaneously, in addition to the sinus glands, the four large ganglionic nerve masses in each stalk, as well as the so-called X-organ which has been suspected of secretory function. Removal of the sinus gland alone is now feasible by methods which have been described by Brown (1942), by Panouse (1946) and by Kleinholz (1947). The last-named method by which the sinus gland and its large nerve can be readily exposed was used in excising the gland with little or no damage to the optic ganglia, and also to denervate the gland.

Results of Asphyxia

In the first of these studies (Kleinholz and Little, 1949), it was reported that asphyxia in Libinia, obtained by removing the animals from sea water and exposing them to air for an hour, caused a marked hyperglycemia, the resulting concentration of blood-sugar being 2 to 3 times that before the asphyxiating treatment. The earlier-mentioned report of Abramowitz, Hisaw and Papandrea (1944) suggested to us the possibility that the sinus gland might be concerned in the regulation of blood-sugar concentration. Exploration of the possibility in Libinia added further credence to this view when it was found that individuals from which both sinus glands had been removed by excision of the eyestalks no longer showed the marked hyperglycemia of asphyxia demonstrated by normal individuals. This evidence could not, however, be considered as conclusively demonstrating that the sinus gland was responsible for the glycemic effect because, as has been explained above, eyestalk ablation removes a considerable amount of other tissue. The effect of asphyxia was, therefore, re-examined in the present study in normal animals and in animals in which only the sinus glands had been excised or denervated.

TABLE I

The effect of asphyxia on the concentration of blood-sugar

The concentration of blood-sugar before and after asphyxial treatment is given in milligrams per 100 ml. of blood (mg.%), with the standard deviation from the average of the group; the numerals in parentheses following the concentration after asphyxia indicate the number of animals in the group that showed increases of less than 3 mg.%; *A*, asphyxia attempted by keeping the animals in air; *—ES*, eyestalkless animals; *—SG*, sinus-glandless animals; *—SGN*, animals in which both sinus glands had been denervated; *W*, asphyxia accomplished by maintaining animals in de-aerated water.

Animals		Asphyxia		Average mg.% blood-sugar		Average per cent increase
Condition	No. used	Method	Duration	Before	After	
Astacus:						
Normal	5	A.	2 hrs.	16.5 ± 9.5	15.6 ± 8.1 (4)	0
Normal	5	A.	4 hrs.	19.7 ± 2.4	19.1 ± 2.4 (5)	0
Normal	5	A.	8.5 hrs.	20.0 ± 3.8	18.7 ± 5.5 (5)	0
Normal	10	W.	1 hr.	16.7 ± 7.9	29.0 ± 21.1 (4)	74
—SG	9	W.	1 hr.	14.3 ± 8.9	14.2 ± 8.7 (8)	0
Normal	16	W.	3 hrs.	18.3 ± 6.5	52.9 ± 22.4 (1)	189
—SG	10	W.	3 hrs.	11.6 ± 5.8	12.7 ± 5.1 (8)	9
Callinectes:						
Normal	4	A.	2 hrs.	9.2 ± 1.7	27.7 ± 10.3 (0)	201
—ES	6	A.	2 hrs.	9.7 ± 3.8	8.0 ± 3.2 (6)	0
—SGN	11	A.	2 hrs.	14.4 ± 4.9	16.9 ± 6.5 (6)	17

Attempts to reproduce the hyperglycemia of asphyxia in Astacus by removing the animals from water and exposing them to air, in much the same manner as had been employed with Libinia, were unsuccessful. No appreciable change in concentration of blood-sugar was obtained with Astacus which had been kept for 2, 4, and 8.5 hours in air (Table I) ; in 5 of the 15 animals very slight rises occurred after treatment, two animals showing increases of 4.3 and 4.0 mg.%, while the three other increases ranged between 0.5 and 2.0 mg.% ; in the 10 remaining animals small decreases in blood-sugar values, from 0.6 to 5.5 mg.%, were observed.

A more effective method of asphyxia was found by immersing individuals in water from which air had previously been removed by vacuum. When normal Astacus were immersed for 1 hour in such de-aerated water, the effect on the concentration of sugar in the blood was variable: 4 of the 10 treated animals in Table I showed very marked increases, the blood-sugar concentration being 2 to 3½ times the concentration before asphyxia was begun; 2 crayfish showed lesser rises, while the remaining 4 individuals of this group showed small decreases in concentration ranging from 0.8 to 2.7 mg.%. The average increase for this group as a result of asphyxia was 74 per cent. When 9 Astacus from which the sinus glands had been removed were tested in the same way, the group average for blood-sugar concentration after asphyxia was not significantly different from the average before treatment. In 2 of these animals there was a post-asphyxial increase in blood-sugar concentration amounting to 5.7 and 1.7 mg.%, while in 6 animals slight decreases ranging from 0.4 to 2.3 mg.% occurred.

To see whether more uniform hyperglycemic responses could be obtained, groups of normal crayfish were asphyxiated as before, but the exposure was increased to 3 hours. Of 16 animals thus treated, 15 showed post-asphyxial concentrations of blood-sugar that were from 1½ to 6 times greater than the pre-treatment glycemic values, while one animal showed a decrease of 5.4 mg.%. The average increase as a result of asphyxia was 189 per cent. Ten sinus-glandless Astacus which were exposed for 3 hours to the same anoxic conditions showed an average rise of 1.1 mg.% in blood-sugar concentration. Of these 10 animals the distribution of change was as follows: 2 showed increases of 7.7 and 8.2 mg.%, 3 showed slight increases between 1.0 and 2.0 mg.%, while 5 showed slight decreases ranging from 1.0 to 2.7 mg.%.

With Callinectes the hyperglycemia of asphyxia could be obtained by using the same method employed with Libinia, *i.e.,* removing the animals from a water to an air environment. Blood-sugar concentrations in normal crabs after 2 hours in air were 2 to 4 times the pre-asphyxial level, with an average increase of 201 per cent. No hyperglycemia occurred in 6 Callinectes which were asphyxiated after ablation of both eyestalks; 5 of these animals showed insignificant decreases in glycemic level, the maximum being 1.2 mg.%; the average decrease of 18 per cent for the group is due to a very marked drop of 8.2 mg.% in the sixth animal.

An experiment of additional interest was performed with Callinectes in which the relatively large sinus gland nerves were cut. In 2 animals bilateral denervation of the gland was done 11 days before and in the remaining 9 animals from 12 to 36 hours before the beginning of the experiment. The group average for blood-sugar concentration after asphyxia showed an increase of 17 per cent, in striking contrast to the 201 per cent increase obtained with normal individuals after similar treatment. Whether this 17 per cent increase is significant, in view of the relatively small number of animals used in the experiment, is difficult to determine. Four individuals of this group which had been operated 11 days, 36 hours, 16 hours, and 12 hours before beginning the experiment showed increases in the glycemic concentration respectively of 1.8, 0.6, 0.6, and 2.7 mg.%; 5 Callinectes showing somewhat larger increases, 8.0, 7.0, 6.4, 3.5, and 3.1 mg.% had been operated, respectively, 16, 14, 23, 18, and 21 hours earlier; 2 crabs which showed decreases of 2.3 and 3.6 mg.% had been 11 days and 12 hours, respectively, since the operation of denervation. There thus seems to be no definite relation between the recency of surgical procedure and the slight hyperglycemia produced by asphyxia in the animals with denervated sinus glands. Denervation of this gland prevents the considerable hyperglycemia that appears in asphyxiated normal animals and indicates the participation of the central nervous system in producing this effect. A possible explanation of the slight rise in glycemic level in animals with denervated sinus glands will be considered below in the discussion of the possibility of sources of hormone outside these glands.

Results of Anesthesia

The readiness with which crustaceans responded by a rise in blood-sugar concentration to crowding and handling (Abramowitz, Hisaw and Papandrea, 1944), and to asphyxia (these studies), led us to suspect that this response might be analogous with the excitement hyperglycemia of mammals. Since a number of investigators have reported that surgical anesthesia in mammals produces marked hyperglycemia, we sought to determine whether chloroform anesthesia would similarly effect an increase in the blood-sugar concentration of Astacus.

Table II

Effect of chloroform anesthesia on blood-sugar concentration in Astacus

Animals		Anesthesia		Average mg.% blood-sugar		Average per cent increase
Condition	No. used	Concentration	Duration	Before	After	
Normal	11	1:1750	1 hr.	15.9 ± 4.1	21.6 ± 5.2 (3)	36
Normal	11	1:1750	1 hr.	36.2 ± 10.8	67.5 ± 12.4 (0)	86
—SG	16	1:1750	1 hr.	16.0 ± 5.6	16.6 ± 5.2 (11)	4
Normal	18	1:830	1.5 hrs.	24.0 ± 8.8	38.0 ± 8.6 (3)	58
—SG	7	1:830	1.5 hrs.	12.2 ± 5.7	12.3 ± 8.3 (5)	1
Normal	17	1:830	2.0 hrs.	24.1 ± 7.3	72.5 ± 28.2 (0)	200

The necessary concentration and duration of treatment with chloroform were worked out by trial from the known experience (Kleinholz, 1947) that exposure of the crayfish, Cambarus, for 2 to 3 minutes to a concentration of approximately 1 : 500 of chloroform in water was adequate for surgical anesthesia. With less concentrated solutions we succeeded in anesthetizing animals and maintaining them in this state for 1 to 2 hours with complete recovery following their return to flowing water. The effects of such anesthesia on the concentration of blood-sugar in normal Astacus and in individuals from which both sinus glands had been removed are summarized in Table II.

In the first group, with a concentration of 1 : 1750, 3 of the 11 animals tested showed little increase in glycemic level, while the remaining 8 Astacus showed individual increases in blood-sugar concentration ranging from 4.8 to 10.7 mg.% as a result of the chloroform treatment. The average increase of 36 per cent, while marked, is not as striking as the increases obtained by asphyxia in the preceding section.

A second group of 11 crayfish differed from the first group in that the animals had not been starved before the experiment; as a result the concentrations of blood-sugar were relatively high. This group demonstrated a more striking hyperglycemia after anesthesia, even when treated in the same manner as the first group with respect to duration of exposure and concentration of the chloroform solution. The individual increase in these after anesthesia was not less than 12 mg.%, the glycemic levels ranging from 1½ to 3 times those before anesthesia; the average increase of 86 per cent is considerably higher than that obtained with the first of these groups.

The difference in hyperglycemic response between the two similarly treated groups may be due to a greater carbohydrate reserve in the case of the fed animals, thus making possible a greater rise in glycemic concentration, or it may be due to individual differences in sensitivity to chloroform. The possibility of this latter factor is indicated by the differences in the degree of hyperglycemia shown by two other groups of normal Astacus which were immersed in more concentrated chloroform solution (1 : 830). In one group of 18 Astacus the average increase was 14 mg.%. In the second group consisting of 17 individuals treated with the same concentration of chloroform, and having an average blood-sugar concentration before anesthesia practically identical with the preceding group, an increase of 30 minutes in the time of exposure to chloroform resulted in a much more striking hyperglycemia. All the individuals showed glycemic values that were from 1½ to 9 times the concentration before treatment; the average increase in this second group was 200 per cent, compared with the 58 per cent average increase shown by the former group. The degree of hyperglycemia may thus depend on the concentration of the anesthetic and the duration of exposure to it.

The effect of chloroform anesthesia on animals from which both sinus glands had been removed was quite different from the responses of the normal crayfish. When 16 sinus-glandless animals were exposed for 1 hour to 1 : 1750 chloroform, 8 showed either no change or the minor fluctuations of plus or minus 1 mg.%, 5 showed an increase in blood-sugar concentration ranging from 3.0 to 6.4 mg.%, and in the remaining 3 decreases of 4 to 5 mg.% occurred. The average concentration of blood-sugar after chloroform treatment was only 4 per cent higher than the average concentration before treatment, as compared with the glycemic increases of 36 per cent and 86 per cent shown by normal animals under similar conditions.

A second group of 7 sinus-glandless animals was similarly tested with the higher concentration of chloroform water (1 : 830) for one and one-half hours. Two crayfish showed increases of 3.1 and 5.6 mg.%, three showed slight decreases in glycemic level ranging between 2.0 and 4.6 mg.%, while the remaining two individuals showed no change. The average glycemic level after anesthesia was less than 1% higher than the initial concentration of blood-sugar.

It may thus be concluded that the presence of the sinus glands is necessary for the marked hyperglycemia of anesthesia to occur.

Results of Adrenaline Injection

We decided to re-study the effect of adrenaline on glycemic level in crustaceans, not so much for the analogy with its hyperglycemic effect on vertebrates, but because the sinus gland is prominently innervated. Hanström (1937) and Welsh (1941) have described the sinus gland nerve as originating in the large optic ganglion, the medulla terminalis; in Cambarus, Welsh has found additional fibers originating in the supra-esophageal ganglion which join those from the medulla terminalis to constitute the sinus gland nerve. Evidence from our experiments in Callinectes whose sinus gland nerves had been transsected, indicated that the hyperglycemia of asphyxia was mediated through stimuli that passed to the gland by way of this nerve. It

TABLE III

Effect of injection of adrenaline and of control substances on the concentration of blood-sugar

Animals		Injection		Average mg.-% blood-sugar		Average per cent increase
Condition	No. used	Substance	Amount	Before	After	
Astacus:						
Normal	5	adrenaline	100γ	14.1 ± 8.3	42.0 ± 13.7 (0)	198
Normal	32	adrenaline	50	11.0 ± 4.5	29.6 ± 9.3 (0)	169
Normal	36	adrenaline	20	12.0 ± 3.6	21.9 ± 10.7 (12)	83
Normal	22	adrenaline	10	12.8 ± 3.4	20.7 ± 10.3 (9)	62
Normal	15	adrenaline	5	8.3 ± 2.8	11.9 ± 4.6 (7)	43
Normal	14	adrenaline	2	13.2 ± 4.9	18.9 ± 8.5 (6)	43
Normal	9	adrenaline	1	15.3 ± 4.2	20.2 ± 6.2 (5)	32
Normal	11	water	0.2 ml.	10.1 ± 3.3	10.1 ± 4.2 (10)	0
Normal	12	chloretone	0.1 ml.	13.1 ± 3.8	12.8 ± 4.4 (9)	0
—SG	5	adrenaline	50	6.3 ± 2.1	5.8 ± 3.8 (4)	0
—SG	6	adrenaline	100	*4.8 ± 1.4	*7.5 ± 4.1 (2)	56
—ES	8	adrenaline	100	12.2 ± 2.6	9.9 ± 2.0 (8)	0
—SG	7	adrenaline	100	14.2 ± 2.8	15.7 ± 6.4 (4)	11
Callinectes:						
Normal	6	adrenaline	150	20.5 ± 2.6	45.4 ± 4.8 (0)	121
Normal	4	adrenaline	50	8.6 ± 3.4	37.4 ± 12.1 (0)	335
Normal	7	adrenaline	10	11.2 ± 4.4	29.8 ± 11.8 (0)	105
Normal	9	adrenaline	1	13.5 ± 5.1	26.7 ± 7.0 (0)	98
Normal	5	adrenaline	0.1	18.9 ± 7.0	36.6 ± 11.5 (0)	94
Normal	12	saline	0.2 ml.	14.9 ± 4.9	29.4 ± 8.0 (2)	97
—SGN	6	adrenaline	150	15.6 ± 3.6	56.7 ± 20.9 (0)	263
—SGN	4	adrenaline	50	8.4 ± 1.3	27.6 ± 11.9 (0)	229
—SGN	4	adrenaline	10	18.6 ± 1.6	29.8 ± 7.9 (1)	60
—SGN	6	saline	0.2 ml.	10.4 ± 2.7	11.2 ± 4.5 (5)	8
—SG	5	adrenaline	150	10.8 ± 3.0	39.8 ± 10.1 (0)	269
—SG	5	adrenaline	50	18.7 ± 6.1	41.4 ± 10.3 (0)	121
—SG	5	adrenaline	10	14.3 ± 13.4	23.9 ± 8.7 (1)	67
—ES	5	adrenaline	150	14.2 ± 6.1	33.0 ± 11.4 (0)	132
—ES	4	adrenaline	50	12.6 ± 3.7	25.4 ± 12.7 (0)	102
—ES	5	adrenaline	10	15.4 ± 7.1	18.2 ± 8.6 (3)	18
—ES	4	saline	0.2 ml.	13.5 ± 4.0	11.3 ± 1.9 (4)	0

* See text.

appeared that adrenaline, or some closely related compound, might be acting at the nerve terminals in the sinus gland to induce hyperglycemia. We, therefore, examined the effect of injecting adrenaline in a series of doses ranging from 150 γ to 0.1 γ.

In the first group of normal Astacus into which 100 γ of adrenaline were injected, all 5 animals showed a marked hyperglycemia after 90 minutes; the individual glycemic values were from 1½ to 7 times the pre-injection concentration of blood-sugar, while the average increase was 198 per cent. Injection of 50 γ of adrenaline into 32 animals similarly resulted in a rise in blood-glucose in all the individuals, the post-injection concentrations ranging from 1½ to 10 times those before injection, and the average increase being 169 per cent.

With injection of lesser amounts of adrenaline, as shown in Table III, the individuals of each group became more variable in their responses. Thus, of 36 animals injected with 20 γ of adrenaline, 7 showed slight increases ranging between 0.1 and 2.4 mg.%, 5 showed slight decreases varying between 0.9 and 4.9 mg.%, while the remaining 24 individuals demonstrated rises in blood-sugar concentration from 4.5 to 36 mg.% ; the average increase over the pre-injection glycemic value was 83 per cent. Smaller quantities of adrenaline (injection of 10, 5, 2, and 1 γ) resulted in even more marked variability in response; in approximately half the number of animals of each group thus injected no appreciable increase in blood-sugar concentration occurred, while in the remainder sufficient rises in glycemic level took place to yield average increases of 62%, 43%, 43%, and 32%, respectively.

Eleven Astacus were injected with 0.2 ml. of distilled water as controls to the above experiments. Although the average for the group was the same after injection as before, the slight individual fluctuations observed in the preceding groups were also present here. Increases in glycemic concentration between 0.2 and 2.4 mg.% occurred in 4 crayfish; one animal had an increase of 3.5 mg.% ; and 6 Astacus showed decreases ranging from 0.4 to 2.5 mg.%. Similar lack of hyperglycemic effect was found on injection of 0.1 ml. of saturated chloretone solution into 12 Astacus; chloretone was used as a control because the commercial adrenaline was dissolved in 0.5 per cent solution of this substance. In 8 of these injected animals decreases in blood-sugar concentration from 0.2 to 4.3 mg.% took place, while 4 crayfish showed increases ranging from 1.6 to 7.1 mg.%.

The hyperglycemic effects of adrenaline on the crayfish of this study thus seem to be unquestionable; very pronounced increases in blood-sugar concentration were obtained with doses ranging from 100 γ to 10 γ; smaller average increases were obtained with lesser doses. Injection of control substances was without appreciable effect on glycemic levels.

Several possibilities have suggested themselves as to the site of action of injected adrenaline; one, mentioned at the beginning of this section, is that adrenaline may be acting as a neurohumoral mediator, either in the central nervous system of the crustacean, or between the sinus gland nerve and the sinus gland; a second possibility is that adrenaline more or less directly effects release of glucose from an organ in which reserve carbohydrate is stored. Because of the failure of asphyxia and anesthesia to produce appreciable hyperglycemia in Astacus from which the sinus glands had been removed, as contrasted with the pronounced increases in normal animals, we decided to test the first of these possibilities by injecting adrenaline into sinus-glandless Astacus.

Five sinus-glandless crayfish were injected with 50 γ of adrenaline. In two animals increases of 0.9 and 3.7 mg.% appeared, while the remaining 3 individuals showed decreases in blood-sugar concentration of 3.2, 2.7, and 1.2 mg.%. The average blood-sugar concentration for the 5 animals, however, showed little change from that before injection, in contrast to the increase of 169 per cent obtained with identical treatment of normal crayfish. Injection of a higher dose of adrenaline, 100 γ, into one group of 6 sinus-glandless Astacus resulted in small increases of 3.9 to 6.9 mg.% in 4 animals and decreases of 2.0 and 2.7 mg.% in two. The average blood-sugar concentrations before and after injection are shown in Table III; there was an average increase of 56 per cent in glycemic concentration. While at first glance this rise of 56 per cent in animals without sinus glands might indicate the presence of a source of diabetogenic hormone other than the sinus gland, explanation of this increase may lie in other directions.

For example, this increase may have resulted from the accumulation, in the small group of experimental animals employed, of individual variations that were magnified by the low pre-injection glycemic values. We might interpolate the fact that a higher than usual set of blanks in the titration of this set of blood samples resulted in the low initial concentration of 4.8 mg.%, although the volume of ceric sulfate used in titrating the blood samples was about the same as that which usually indicated glucose concentrations between 10 and 12 mg.%. Other explanations of this same increase may, however, lie in partial regeneration (or incomplete removal) of the sinus glands, or in the action of adrenaline directly on a carbohydrate storage depot without the mediation of another endocrine secretion. To test between some of these possibilities, 8 Astacus, from each of which both eyestalks had previously been ablated, were injected with 100 γ of adrenaline. In 6 animals decreases in blood-sugar concentration (from 1.7 to 5.5 mg.%) and in 2 slight increases (1.0 and 1.4 mg.%) occurred, the average for the group being a decrease of about 19 per cent. The results do not support the possibility that adrenaline in Astacus was effecting release of glucose from a carbohydrate-storing organ. While these same results rule out the possibility of a diabetogenic hormone originating outside the eyestalk of the crayfish, they do not exclude some structure, other than the sinus gland, in the eyestalk from being a source of hyperglycemic hormone. The experiment was, therefore, repeated with a second group of 7 sinus-glandless Astacus which were injected with 100 γ of adrenaline to determine whether an increase approximating the 56 per cent rise obtained with the first group would be repeated, or whether the increase in this first group had been due to fortuitous variation. Three animals showed decreases (from 2.1 to 5.9 mg.%) while 4 showed increases (0.2, 3.4, 8.8, and 9.7 mg.%), the average for the group being a rise of 11 per cent. We are, therefore, inclined to propose, tentatively, the latter explanation of the glycemic increase in the first of these sinus-glandless groups injected with 100 γ of adrenaline. We do not believe that other factors suggested above as explanations of the increase have been decisively excluded, especially since two of the crayfish in this second group showed rather marked glycemic increases (of 8.8 and 9.7 mg.%); further study is required of this point.

Whatever may be the explanation of the slight hyperglycemia effected in sinus-glandless Astacus, comparision of the effects of adrenaline injection in normal crayfish with those of animals without sinus glands indicates quite convincingly that the adrenaline-induced hyperglycemia is mediated by way of the sinus gland.

Adrenaline injection in Callinectes was followed, in general, by hyperglycemias in normal crabs similar to those resulting in Astacus. There were, however, some interesting differences. A series of doses of adrenaline, from 150 to 0.1 γ (Table III), brought about an average increase of 100 per cent or more in glycemic concentration. A striking unanimity of response was observable in the members of each injected group, unlike the prominent variability that occurred with similar treatment in Astacus; this was especially marked with the lower dosages of adrenaline. Suspicion of this unanimity was justified when it was found that 0.2 ml. of 0.7 per cent NaCl injected into normal Callinectes was as equally effective as the lower doses of adrenaline in evoking about a 100 per cent increase in blood-sugar concentration. We believe that this is illustrative of excitement hyperglycemia due to the handling of the animals in taking blood samples and making injections; Callinectes is much more aggressive than Astacus and apparently responds more readily than does the crayfish to these manipulations by exhibiting a marked hyperglycemia; there is no reason to suspect saline solution *per se* of any effect on blood-sugar concentration.

Confirmation of this view was obtained with the next sequence of experiments on Callinectes in which the nerves to the sinus glands had been severed bilaterally from 1 to 14 days preceding the experiment. Injection of 150 and 50 γ of adrenaline into groups of Callinectes whose sinus gland nerves had been cut 2 to 3 days and 13 to 14 days respectively brought about a net increase of more than 200 per cent in blood-sugar concentration; 10 γ of adrenaline injected into a group whose sinus gland nerves had been cut 2 to 8 days previously evoked a much smaller average increase. Injection of saline into similar animals in which the nerves had been cut 1 to 3 days yielded no significant increase in blood-sugar concentration. We, therefore, believe that the marked hyperglycemias resulting from injection of saline and of the smaller doses of adrenaline in the normal crabs were due to secretion of sinus gland hormone effected through the sinus gland nerves; the very great increases in glycemic levels obtained with the higher doses of adrenaline (150 and 50 γ) we might then attribute to the neurohumoral action of adrenaline on the denervated gland.

The inadequacy of this last interpretation was, however, demonstrated by a series of experiments whose results are in striking contrast to those obtained in practically identical tests performed with Astacus. Three groups of Callinectes whose sinus glands had been removed from 2 to 11 days preceding the experiment were injected with 150, 50, and 10 γ of adrenaline, respectively. It will be recalled from experiments described above that injection of adrenaline into sinus-glandless Astacus was attended by a modest rise in blood-sugar concentration of some individuals, no conclusive explanation for this rise being proposed at the time. Similar treatment of sinus-glandless Callinectes, however, resulted in an impressive increase in glycemic level. Hence, the explanation that adrenaline-induced hyperglycemia in animals with denervated sinus glands might be due to the action of this drug as a chemical mediator on the gland is not wholly adequate. Since this last series of experiments demonstrates a convincing hyperglycemia in animals without sinus glands, additional interpretation is not excluded. We are consequently faced with considering the presence of a mechanism in the adrenaline-induced hyperglycemia by which some structure in the eyestalk, other than the sinus gland, might be involved in the hyperglycemic response. The X-organ, a structure whose appearance of secretory activity had already impressed Hanström (1931), who originally described it in considerable detail, is one such possibility, although others may also be present.

We, therefore, explored the possibility that, in addition to the methods described above, adrenaline might be evoking hyperglycemia by acting on the X-organ or some hitherto as yet unrecognized glandular tissue in the eyestalk. Injection of the usual doses of adrenaline into eyestalkless crabs resulted in striking hyperglycemias with the two higher concentrations (150 and 50 γ) and only a very slight increase in blood-sugar after the 10 γ dose. The average percentage increases in glycemic level after the 150 and 50 γ injections were not as high as in preceding groups of experiments, but we doubt that this difference can be considered significant in view of the small number of animals in each group.

We can, therefore, conclude from the qualitative nature of the latter series of tests 1) that injection of adrenaline in Callinectes (and in Astacus) results in hyperglycemia; 2) that while hyperglycemia is effected through the sinus gland (anesthesia and asphyxia experiments with Astacus and Callinectes), adrenaline can also evoke hyperglycemia (convincingly in Callinectes, but much less clearly so in Astacus) in the absence of the sinus glands. Whether adrenaline may thus be acting on glandular tissue outside of the eyestalk to result in release of diabetogenic hormone, or whether adrenaline may be acting directly on tissue in which carbohydrate is stored to result in release of glucose, is as yet not known. Some of our preliminary experiments to explore these last hypotheses were too few and the results too equivocal to be discussed at this time.

Discussion

The experiments described above demonstrate the necessity of the sinus gland for asphyxia- and anesthesia-induced hyperglycemia to occur. Failure of hyperglycemia to appear after asphyxia in animals whose sinus gland nerves had been cut indicates that the response is part of a reflex mechanism. The details of the mechanism of chloroform-induced hyperglycemia were not explored beyond indicating its dependence on the presence of the sinus gland; study of the sequence of effect of anesthesia on the centers of the crustacean nervous system could not be critically assessed at this time and were beyond the scope of this investigation. The appearance of Needham's (1950) paper supplies some of this information. This author found that the neurogenic heart of decapod crustaceans is arrested by ether and chloroform anesthesia earlier than are the somatic movements; complete quiescence of the heart for periods up to an hour was not attended by fatal consequences, recovery, in the case of Carcinus, taking 1 to 2 hours. The circulatory stasis accompanying this cardiac arrest must build up a high oxygen debt. The effect of chloroform anesthesia on the sinus gland may thus be mediated by the same centers of the nervous system that are involved in the hyperglycemia resulting from asphyxia.

Conclusive evidence for the activity of chemical mediators of nervous transmission in crustacea has not been abundant. Some studies (for review see Bacq, 1947) reporting the presence of such mediators have been acute experiments involving perfusion of isolated organs, or the injection of acetylcholine or adrenaline into the organism, as was done with the latter substance in this study. Experimental investigations of this nature are actually little more than reports of the pharmacological effect of the injected substances; they do not prove the role of such substances as chemical mediators in the physiology of the organism, but indicate only the possibility of such a role. Prosser (1942) has presented criteria to be observed in considering the physiological role of acetlycholine. Comparable standards could be established to define the relation between adrenaline and the presence of adrenergic nerves. Bacq has in fact reviewed critically a number of studies concerned with the effect of acetylcholine and sympathomimetic amines among invertebrates.

Bacq's view that invertebrate tissues show a non-specific and often low sensitivity to adrenaline and the sympathomimetic amines might be modified in the light of more recent information. His view was based in part on the contradictions in the literature, already summarized in the introduction above, on the hyperglycemic effect of adrenaline in crustacea. When Kalmus and Waldes reported in 1936 that the hyperglycemic response in crustaceans was non-specific, being just as readily evoked by injection of physiologically irrational substances as by adrenaline, the importance of the sinus gland as an endocrine organ and its being a source of diabetogenic hormone was not yet known. The demonstration in this study of the role of the sinus gland in excitement hyperglycemia may explain the results of Kalmus and Waldes; thus, their questioning of the specificity of the adrenaline-induced hyperglycemia would become instead a question as to whether these non-specific substances were effecting hyperglycemia by acting through the nervous system to bring about release of diabetogenic hormone from the sinus glands.

Bacq's point concerning the low sensitivity of invertebrate tissues to adrenaline might not seem applicable to the adrenaline-induced hyperglycemia described here. The lower threshold of hyperglycemic doses of adrenaline for the Astacus of this study was between 1 and 5 γ. If, for conveniently simplifying the calculations, we consider the crayfish used to have weighed 100 grams, the blood-volume of such animals would be about 25 ml., according to the determinations of Prosser and Weinstein (1950) on Cambarus. If the injected adrenaline is uniformly mixed and persists *in toto* in the blood without being partially eliminated or inactivated, the concentration of adrenaline in the vascular system of the animal that brings about an appreciable increase in the glycemic level is then about 10^{-7}. Sensitivity to such concentrations of adrenaline is not far removed from that shown by some isolated organs or tissues used in the assay of chemical mediators of nervous transmission.

It is obvious that isolated observations, such as those above, do not constitute adequate evidence for the existence of adrenergic nerves in crustacea, but they do remove some of the criticisms that had been levelled against such observations in the past. Criteria for conclusive proof of the existence of adrenergic nerves can be established with which to compare results from future experimental procedures aiming toward this end.

SUMMARY

1. Asphyxia of normal Astacus and Callinectes results in marked increase in concentration of blood-sugar; such increase is mediated by sinus glands located in the eyestalks, for after removal of these glands hyperglycemia does not occur after asphyxia. Hyperglycemic response to asphyxia is part of a reflex mechanism; it does not occur following denervation of the sinus glands.

2. Chloroform anesthesia in Astacus similarly evokes rises in glycemic level which fail to occur after sinus gland removal. Since anesthesia brings about cardiac arrest, the resultant anoxia may be the basic explanation of the anesthesia-induced hyperglycemia.

3. Adrenaline, in doses from 1 to 100 γ, causes hyperglycemia in normal Astacus, but injection into sinus-glandless crayfish shows either no rise in the concentration of blood-sugar, or a very slight one. Similar injections into Callinectes result in marked hyperglycemia in normal individuals, in animals with denervated sinus glands, in sinus-glandless and also in eyestalkless individuals.

4. The results of this study indicate the presence of an excitement hyperglycemia in these crustaceans, ensuing from release of diabetogenic hormone from the sinus glands. The effects of adrenaline in Callinectes may be due to its action on glandular tissue outside of the eyestalk, bringing about secretion of diabetogenic hormone

(in addition to its effect on the sinus glands), or to its action on carbohydrate-storing tissue resulting in release of glucose.

Literature Cited

Abramowitz, A. A., F. L. Hisaw and D. N. Papandrea, 1944. The occurrence of a diabetogenic factor in the eyestalks of crustaceans. *Biol. Bull.*, **86**: 1–5.

Bacq, Z. N., 1947. L'acétylcholine et l'adrénaline chez les invertébrés. *Biol. Rev.*, **22**: 73–91.

Brown, F. A., Jr., 1942. Sinus gland extirpation in the crayfish without eyestalk removal. *Proc. Soc. Exper. Biol. Med.*, **50**: 295–297.

Brown, F. A., Jr., 1948. Hormones in Crustaceans. *In* The Hormones, Vol. I, 159-199. Academic Press, New York.

Florkin, M. and G. Duchateau, 1939. La glycémie de l'écrevisse apres l'injection d'adrénaline ou d'insuline. *Compt. Rend. Soc. Biol. Paris*, **132**: 484–486.

Hanström, B., 1931. Neue Untersuchungen über Sinnesorgane und Nervensystem der Crustaceen. I. *Zeit. Morph. u. Ökol. Tiere*, **23**: 80–236.

Hanström, B., 1937. Die Sinusdrüse und der hormonal bedingte Farbwechsel der Crustaceen. *Kungl. Svenska Vetenskap. Handl.*, Ser. 3, **16**: 1–99.

Hemmingsen, A. M., 1924. Blood-sugar regulation in the crayfish. *Skand. Arch. Physiol.*, **46**: 51–55.

Kalmus, H. and V. Waldes, 1936. Ist die durch Adrenalin bewirkte Glycolyse beim Flusskrebs spezifisch? *Zeit. vergl. Physiol.*, **23**: 712–714.

Kleinholz, L. H., 1942. Hormones in Crustacea. *Biol. Rev.*, **17**: 91–119.

Kleinholz, L. H., 1947. A method for removal of the sinus gland from the eyestalks of crustaceans. *Biol. Bull.*, **93**: 52–55.

Kleinholz, L. H. and B. C. Little, 1949. Studies in the regulation of blood-sugar concentration in crustaceans. I. Normal values and experimental hyperglycemia in *Libinia emarginata*. *Biol. Bull.*, **96**: 218–227.

Lindblad, R. G., 1931. Studien über den Blutzucker bei Astacus fluviatilis. Kristianstad.

Medvédéva, Nat., 1936. Le problème de la réactivité spécifique des invertébrés aux increts des vertébrés. IV. Action de l'adrénaline et de l'insuline sur certains invertébrés. *Medichnii Zhurnal vseukrain. Acad. U.S.S.R.*, Kiev, **6**: 385–387.

Medvédéva, Nat., 1936. Le problème de la réactivité spécifique des invertébrés aux increts des vertébrés. Du mechanisme de la régulation du sucre de l'hémolymphe chez différents invertébrés sous l'action de l'adrénaline et de la surcharge glucosée. *Medichnii Zhurnal vseukrain. Acad. U.S.S.R.*, Kiev, **6**: 805–807.

Miller, B. F. and D. D. Van Slyke, 1936. A direct microtitration for blood-sugar. *Jour. Biol. Chem.*, **114**: 583–595.

Needham, A. E., 1950. The neurogenic heart and ether anesthesia. *Nature*, **166**: 9–11.

Panouse, J. B., 1946. Recherches sur les phénomènes humoraux chez les crustacés. *Ann. Inst. Océan.*, **23**: 65–147.

Prosser, C. L., 1942. An analysis of the action of acetylcholine on hearts, particularly in arthropods. *Biol. Bull.*, **83**: 145–164.

Prosser, C. L. and S. J. F. Weinstein, 1950. Comparison of blood volume in animals with open and with closed circulatory systems. *Physiol. Zool.*, **23**: 113–124.

Roche, J. and C. Dumazert, 1935. Sur la glycémie de Cancer pagurus. *Compt. Rend. Soc. Biol. Paris*, **120**: 1225–1227.

Stott, F. C., 1932. Einige vorlaüfige Versuche über Veränderungen des Blutzuckers bei Dekapoden. *Biochem. Zeit.*, **248**: 55–64.

Welsh, J. H., 1941. The sinus glands and 24-hour cycles of retinal pigment migration in the crayfish. *Jour. Exper. Zool.*, **86**: 35–49.

GLYCOGEN DEPLETION DURING STARVATION IN THE NEMERTEAN, MICRURA LEIDYI (VERRILL), AND ITS ECOLOGICAL SIGNIFICANCE[1]

W. MALCOLM REID

Monmouth College, Monmouth, Illinois, and The Marine Biological Laboratory, Woods Hole, Mass.

Nemerteans, free-living organisms sometimes popularly known as ribbon-worms or proboscis-worms, are able to survive long periods of complete starvation under aerobic conditions. Coe (1943) reported the survival of some species of Lineus, Prostoma and Procephalothrix for more than a year. During this time the body was greatly reduced in size by the absorption of tissues from the gonads, digestive system and proboscis. Incidental observations were made by Jacubowa and Malm (1931) on worms identified as "Nemertinae" under anaerobic conditions. They noted survival for more than five days in a fouled, oxygen-poor aquarium. In his review of anaerobiosis, von Brand (1946) indicated that this may not have been an oxygen-free environment. He further emphasized the paucity of knowledge of the metabolism of various groups of invertebrates. This lack of information is especially obvious in the phylum Rhynchocoela (Nemertea) where no studies besides those reviewed above have been conducted.

While comparing the glycogen metabolism of various species of parasitic helminths, sufficient numbers of *Micrura leidyi* (Verrill), 1892, were available to study the glycogen metabolism in worms: (1) analyzed directly after collection from the ocean, (2) starved under aerobic conditions for 16 to 35 days, and (3) starved under completely anaerobic conditions for 24 to 72 hours. In addition, observations on other effects of longer anaerobic starvation were made.

MATERIALS AND METHODS

Specimens of *Micrura leidyi* were obtained during July and August by digging in protected sandy bottoms near the low tide mark in the Woods Hole area. The substrate varied from almost pure sand to sandy mud. Other prominent invertebrate organisms in the same ecological association were the holothurian, *Leptosynapta inhaerens,* the molluscans, *Mya arenaria* and *Venus mercenaria,* and several species of polychaete annelids. The nemerteans were transported to the laboratory in individual bottles and experimental procedures initiated as soon as possible. Unfortunately *M. leidyi,* when handled, secretes large quantities of mucus and has a tendency to fragment. As reported by Coe (1943), rapid regeneration of a new caudal cirrus occurs if the anterior end is present. Fragments from the posterior end do not regenerate and soon begin to decay. These disadvantages were counterbalanced by availability and by the convenient size of the organism for weighing and making glycogen determinations on single specimens. Fragmentation and mucus secretion usually ceased within 24 hours after the worm was placed in a container although some specimens continued to fragment and had to be discarded.

---

[1] This study was partially supported by a grant from the American Association for the Advancement of Science awarded by the Illinois State Academy of Science.

For aerobic starvation, worms were placed in filtered sea water in large, covered, crystallizing dishes and stored in a dark room at 18° C. In anaerobic starvation worms were placed in individual bottles of sea water which had been boiled and cooled to 23° C. (temperature of sea water circulated around the bottles). These bottles were stoppered and sealed except for glass tubing connections through which hydrogen or nitrogen was bubbled using the methods of Michaelis and Flexner (1928) to eliminate all traces of oxygen. The assistance of Dr. E. S. G. Barron in making available the apparatus to provide oxygen-free gases is gratefully acknowledged.

Glycogen analyses were made using the combination of methods reviewed by Reid (1942). However, glucose determinations were made with the aid of a photoelectric colorimeter using the standard method of Folin-Malmros.

EXPERIMENTAL

Aerobic starvation. As living worms became available they were divided into three groups. The experimental group was weighed and starved in filtered sea water as long as time at Woods Hole would permit before making a glycogen analysis. The average glycogen level in seven worms was 1.83 per cent after 16 to 35 days of starvation (see Table I). One control group was analysed for glycogen as soon as the worms were dug to determine the food reserve maintained by this animal in its natural environment. Eight worms averaged 1.57 per cent glycogen.

In the original design of the experiment a second control series was set up to study possible deleterious effects of confinement in a smooth, glass vessel. After weighing, the animals were placed in sea water in some of the sand in which they normally burrow. However, three out of four died in a few days. The one survivor, when reweighed after 35 days in the sand, showed a weight loss of 33 per cent as compared to an average loss of 10 per cent in the experimentally starved group. These results are probably caused by loss of a fragment in the sand. Since the experimental group after 35 days in a glass container appeared normal and moved with the usual gliding movement of this species, the second control series was discontinued.

As indicated above, starved worms contained slightly more glycogen than the controls, but this increase is not statistically significant. The fact that there is no decrease during starvation indicates that this organism does not depend upon polysaccharide metabolism during aerobic starvation.

Worms were measured and also weighed to determine decreases in size during the experimental, aerobic starvation. Because of the extensibility of the specimens, length and width measurements proved less reliable than weights obtained on an analytical balance. The loss in weight varied from 3.3 to 17.2 per cent, with an average of 10 per cent for the seven worms. Controls averaged 0.6383 grams total weight at the time of glycogen analysis while experimentals averaged 0.5949 grams.

Anaerobic starvation. During the entire anaerobic period some activity was apparent. The slow gliding movement, probably caused by cilia, could best be detected at the anterior end. Swimming movements are not characteristic of this species and were never observed. One specimen survived seven days of complete anaerobiosis but was dead on the eighth day. Anaerobic conditions frequently induced an increase in fragmentation. Since these pieces decayed and could not be removed without disturbing the condition of anaerobiosis, toxic products may have shortened the survival time. Four specimens were analyzed at the end of 24 hours of anaerobiosis and found to contain 1.49 per cent glycogen. Since this figure was lower, but not low enough to show a statistically significant decrease over the controls, a second series of four worms was starved 72 hours and analyzed. The decrease in glycogen level to 0.29 per cent is statistically significant and indicated an essential role of glycogen in anaerobic starvation of the species. Individual weights of worms averaged 0.7964 grams in the 24 hour group but those starved 72 hours averaged only 0.1488 grams. This average, much lower than the controls, was due to the elimination of fragments before glycogen determinations. Weight loss

TABLE I

Glycogen level in starved and unstarved Micrura leidyi

Treatment	Duration of treatment	Weight loss during treatment	Percentage of glycogen (wet weight)	Average glycogen for group
Unstarved controls analyzed immediately after removal from the ocean (eight worms)	—	—	1.70	
	—	—	1.81	
	—	—	0.88	
	—	—	1.55	
	—	—	1.94	
	—	—	1.62	
	—	—	1.91	
	—	—	1.18	1.57 ± 0.13 (S.E.)
Control kept in sand (one worm)	35 days	33.0%	2.42	2.42
Aerobic starvation (seven worms)	35 days	17.2%	1.53	
	35 days	10.1	1.54	
	23 days	15.2	1.96	
	23 days	4.0	1.50	
	23 days	4.1	2.46	
	23 days	3.3	1.50	
	16 days	15.0	2.32	1.83 ± 0.16
Anaerobic (four worms) starvation	24 hours	not determined	1.10	
	24 hours		1.97	
	24 hours		1.35	
	24 hours		1.53	1.49 ± 0.18
(four worms)	72 hours	not determined	0.17	
	72 hours		0.38	
	72 hours		0.44	
	72 hours		0.16	0.29 ± 0.07

during starvation was not determined because handling prior to initial weighing increased the rate of fragmentation, as mentioned above, and made the worms less suited for anaerobic treatment.

DISCUSSION

The property of enduring long periods of starvation by subsisting on food reserves or tissues is known in a number of invertebrate organisms. Planarians have been the subject of many investigations of this sort. Using histochemical methods Hyman, Willier and Rifenburgh (1924) found no glycogen in *Planaria dorotocephala*. Von Brand (1936) found glycogen in *Planaria torva* which varied from 3 per cent of the wet weight in the Fall to 0.5 per cent during May. This glycogen reserve is reduced to a trace after one week of aerobic starvation. These two studies indicate a less essential role of glycogen and consequently poorer adaptation to anaerobiosis in the economy of this organism than that operative in *M. leidyi.*

In various parasitic helminths the role of glycogen has been under intensive investigation since it is known that large quantities of this polysaccharide are stored by many species. The glycogen reserve, which is much higher in most parasitic forms than in *M. leidyi,* has been interpreted as an adaptation which permits survival under conditions of anaerobiosis in the gut of the host. Hopkins (1950) found as much as 16 per cent of the wet weight of the cestode *Schistocephalus solidus* was glycogen. This reserve is often utilized rapidly under starvation conditions. According to Reid (1942), the cestode *Raillietina cesticillus* used 95 per cent of its stored glycogen when the host was starved 24 hours. Comparison between parasites and free-living forms can be made only in cases where satisfactory *in vitro* techniques of experimentation make possible the control of the oxygen tension during starvation. The larval nematodes, *Eustronglylides ignotus,* utilized glycogen aerobically and more rapidly anaerobically according to von Brand and Simpson (1945).

From the scanty information available, *Micrura leidyi,* with its ability to reserve glycogen for periods of anaerobiosis, may resemble leeches which are reported to have a predominately protein metabolism during starvation. However, as pointed out by von Brand (1946), almost nothing is known of the carbohydrate metabolism in this group. Until this field and the protein metabolism of nemerteans has been investigated no extensive comparison is possible.

Micrura leidyi appears to differ from planarians and the parasitic groups studied by maintaining, under conditions of aerobic starvation, a glycogen reserve which is available to assist in survival under anaerobic conditions. Nemerteans may frequently encounter oxygen-poor conditions for short periods of time in their natural environment. As indicated by von Brand there may be periods of anaerobiosis during low tide for many marine forms. Furthermore, this species is found in burrows deep in sandy-mud where it may be subjected to oxygen-poor conditions. The ability to survive both aerobically and anaerobically without food for relatively long periods is an adaptive mechanism which has probably contributed to the survival of this species.

SUMMARY

1. *Micrura leidyi* maintains a glycogen store of 1.83 per cent of the wet weight of the worm after 16 to 35 days of aerobic starvation. Specimens removed directly

from the ocean had 1.57 per cent glycogen. The ability of an organism to reserve glycogen for use under anaerobic conditions is a possible adaptive mechanism which has not previously been reported.

2. The glycogen level decreased from 1.57 per cent to 1.49 per cent after 24 hours and was further reduced to 0.29 per cent after 72 hours of anaerobiosis.

3. *M. leidyi* is relatively resistant to conditions of complete anaerobiosis. The maximum survival time in the present experiments was seven days.

4. Seven specimens starved aerobically for 16 to 35 days lost an average of 10 per cent of their original body weight.

LITERATURE CITED

COE, W. R., 1943. Biology of the nemerteans of the Atlantic Coast of North America. *Trans. Conn. Acad. Arts and Sci.*, **35**: 129–328.

HOPKINS, C. A., 1950. Studies on cestode metabolism. I. Glycogen metabolism in *Schistocephalus solidus in vivo*. *Jour. Parasitol.*, **36**: 384–390.

HYMAN, L. H., B. H. WILLIER, AND S. A. RIFENBURGH, 1924. Physiological studies on Planaria. VI. *Jour. Exper. Zool.*, **40**: 473–494.

JACUBOWA, L. AND E. MALM, 1931. Die Beziehungen einiger Benthos-Formen des Schwarzen Meeres zum Medium. *Biol. Zentralblatt.*, **51**: 105–116.

MICHAELIS, L. AND L. B. FLEXNER, 1928. Oxidation-reduction systems of biological significance. *Jour. Biol. Chem.*, **79**: 689–722.

REID, W. M., 1942. Certain nutritional requirements of the fowl cestode *Raillietina cesticillus* (Molin) as demonstrated by short periods of starvation of the host. *Jour. Parasitol.*, **28**: 319–340.

VON BRAND, TH., 1936. Studies on the carbohydrate metabolism in planarians. *Physiol. Zool.*, **9**: 530–541.

VON BRAND, TH., 1946. Anaerobiosis in invertebrates. Normandy, Missouri. Biodynamica. 328 pp.

VON BRAND, TH. AND W. F. SIMPSON, 1945. Physiological observations upon a larval Eustrongylides. IX. Influence of oxygen lack upon survival and glycogen consumption. *Proc. Soc. Exp. Biol. and Med.*, **60**: 368–371.

NOTES ON OVULATION, OVA, AND EARLY DEVELOPMENT IN THE SMOOTH DOGFISH, MUSTELUS CANIS[1]

LOIS E. TeWINKEL

Department of Zoology, Smith College and the Marine Biological Laboratory, Woods Hole, Massachusetts

Although there have been a number of studies on development and related problems in the ovo-viviparous *Squalus acanthias* (Scammon, 1911; Ford, 1921; TeWinkel, 1943; Hisaw and Albert, 1947) and in oviparous rays, skates, dogfishes, and sharks (Clark, 1922; Vandebroek, 1936; Metten, 1939; Smith, 1942) there has been almost no recent embryological work on viviparous elasmobranchs other than that treating of utero-gestation (ten Cate-Hoedemaker, 1933; Ranzi, 1932, 1934). This lack of information is readily explained by the difficulty of collecting adequate material. During several summer periods at the Marine Biological Laboratory, Woods Hole, Massachusetts, while collecting embryos of the American smooth dogfish, *Mustelus canis,* for another purpose, certain observations were made on the breeding season, ova, embryonic orientation, and rate of development in this species. These observations serve as the basis for the present report.[2]

Mustelus canis (Mitchell) (Hubbs, 1938) is the only species of Mustelus commonly found along the Atlantic coast of North America. Hubbs considers it to be closely related to the European smooth hound, *M. laevis.* Both species are members of the family *Galeorhinidae* under the order *Galea* (White, 1937). *Mustelus canis* arrives in the vicinity of Woods Hole during May and June and may be collected through August and possibly September, but presumably seeks warmer waters in late autumn. Bigelow and Welch (1924) state that this species is rarely seen north of Cape Cod and that its range extends from Cape Cod to Cuba.

MATERIAL AND METHODS

Mature female specimens of *Mustelus canis,* usually 2½ to 4 feet in length, weighing 5 to 20 pounds, were collected chiefly by fishing from the docks in Woods Hole or from a small boat, provided by Mr. James McInnis of the Supply Department, during the summers of 1945, 1946, 1948, and 1949. Many fishing enthusiasts cooperated in this enterprise but special acknowledgment should be made to Dr. R. T. Kempton who contributed a large number of specimens used in his own experimental work on elasmobranch excretion. Specimens were placed in live cars or in large outdoor aquaria supplied with running sea water. Dr. Kempton's specimens were kept for several days, during which time eggs were occasionally crushed and often extruded. For optimum material fish were opened within twenty-four hours

---

[1] Contribution from the Department of Zoology, Smith College, No. 227.

[2] The genus *Mustelus* has attracted attention embryologically because of the presence of a yolk-sac placenta during much of its extended gestation period (beginning when embryos are 10–16 cm. in length, Ranzi, 1934). In specimens of *M. canis,* collected between June and late August, the placenta had not yet developed.

TABLE I—*Summary of records of embryonic stages in 60 specimens of Mustelus canis*

June			
Date	No. ♀s	No. ut. ova	Notes on embryos
8/45	8	1 in one ♀; none in 7 ♀s	all ♀s pp.; ovaries with 12 to 24 large ova
16/45	1	none	pp., large ova in ovary
25/46	1	6 each uterus	several ova in ovary; st: t.g.d. to bl.
25/49	1	4 each uterus	4 ova in ovary; st: cl. to bl.
27/46	1	1 each uterus	several ova in ovary; st: t.g.d.
28/45	1	5 each uterus	st: cl. to bl.

July			
Date	No. ♀s	No. ut. ova	Notes on embryonic stages
3/45	1	6 each uterus	st: bl. to f.n.f.
3/49	1	7 rt; 6 lft. + 1 empty case ant.	ov. comp.; st: cl. to m.n.f.
7/45	1	7 one uterus; 5 other	st: bl. to 5 mm. embryo
	1	7 one uterus; 8 other	st: cl. to m.n.f.
7/46	1	7 each ut. (d.y. each ut.)	ov. comp.; st: bl. to 8 mm. embryos
7/49	1	5 one ut.; 4 + case ant.	st: f.n.f. to 10 mm. embryos
	1	6 each uterus	st: e.n.f. to 8 mm. embryos
9/46	1	5 one ut.; 6 (1 d.y.) other	ov. comp.; st: 3.5 to 10 mm. embryos
9/49	1	6 each uterus	st: f.n.f. to 11 or 12 mm. embryos
12/48	1	4 one; 2 other (ex.)	ov. comp.; st: bl. to e.n.f.
13/48	1	7 one; 5 other (ex.)	ov. comp.; st: cl. to g.; post. 3 ova crushed
13/49	1	7 each uterus	st: e.n.f. to 10 mm. embryos
14/45	6	No data	st: 2 groups: 1) bl. to n.f. 2) 4 to 12 mm. embryos

July			
Date	No. ♀s	No. ut. ova	Notes on embryonic stages
14/49	1	7 each uterus	st: bl. to 5 or 6 mm. embryos
16/46	1	3 each uterus	st: 10, 13, 18 mm. embryos
16/49	1	9 rt. (1 d.y.) 8 lft.	5 ova in ovary; st: bl. to 7 or 8 mm. embryos
17/46	1	5 each uterus	st: 7 to 15 mm. embryos
18/46	4	approx. 5 each uterus	st: 1 ♀, bl. to 8 mm. embryos; 3 ♀s, 6 to 18 mm. embryos
19/48	1	6 each uterus	st: condition too poor to tell
	1	8 one uterus; 9 other	st: cl. to 3 mm. embryo
20/48	1	7 one uterus; 6 other	st: g. to 8 mm. embryos
21/48	1	4 each uterus	st: f.n.f. to 8 mm. embryos
23/48	1	8 each uterus	st: g. to 10 mm. embryos
24/48	1	7 each uterus	st: 4 to 20 mm. embryos
26/48	1	6 each uterus	st: 6 to 20 mm. embryos
29/48	1	4 one uterus; 5 other	st: 12 to 22 mm. embryos
31/48	1	4 in all (ex.)	st: 13 to 18 mm. embryos
	1	2 one uterus (ex.)	st: 15 and 18 mm. embryos

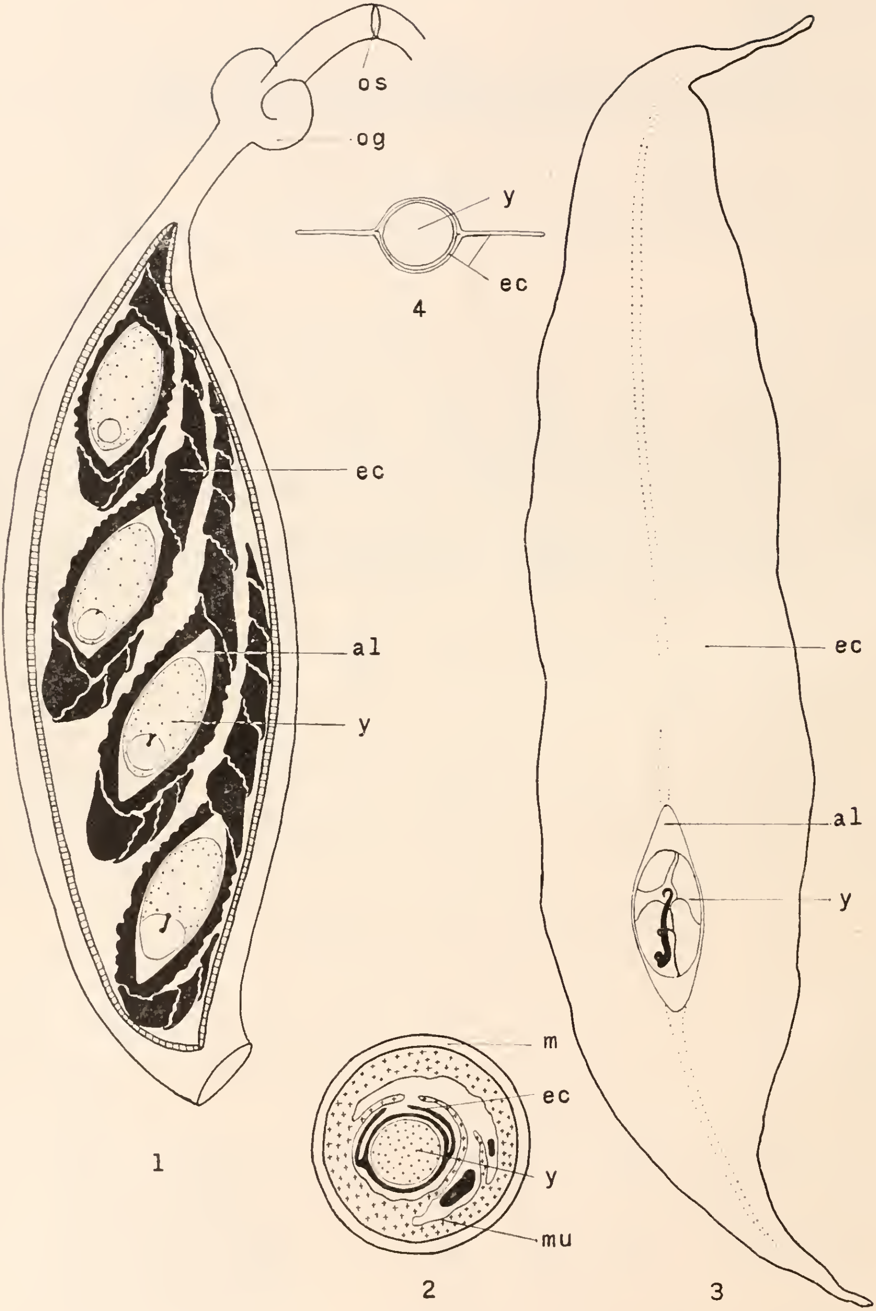
Aug.			
Date	No. ♀s	No. ut. ova	Notes on embryonic stages
1/46	1	5 each uterus	st: 18 to 28 mm. embryos
4/46	1	5 one ut.; 5 empty cases other ut.	st: 15 to 28 mm. embryos
	1	5 each uterus	st: 15 to 25 mm. embryos
4/48	1	8 each uterus	ov. comp.; st: 12 to 27 mm. embryos
9/46	1	no data	st: 28 to 40 mm. embryos
13/48	1	4 each uterus	ov. comp.; st: 57 to 82 mm. embryos†
14/48	1	8 one ut.; 5 other (ex.)	st: 23 to 34 mm. embryos + 1 crushed
16/48	1	6 each uterus	st: 35 to 47 mm. embryos
30/46	2*	total of 6 in 1 ♀; 11 in other	st: approx. 45 to 70 mm.; yolk-sacs nearly empty
31/46	1*	total of 9	st: 50 to 70 mm. embryos; yolk-sacs nearly empty

Key:

bl.	blastodisc
cl.	cleavage
d.y.	"double-yolked" egg
e.n.f.	early neural folds
ex.	extrusion probable
f.n.f.	fusing neural folds
g.	gastrulation stages
m.n.f.	mid-neural folds
ov. comp.	ovulation of large ova completed
pp.	post-partum
st.	embryonic stages
t.g.d.	terminal germinal disc during or before cleavage
ut.	uterus

† Embryos unusually large for date.

* Specimens contributed by Dr. Donald Zinn.


os
og
y
ec
4
ec
al
y
ec
al
y
m
ec
y
mu
1
2
3

Figures 1–4

of collecting; uteri and oviducts were tied off and removed to bowls of sea water for detailed observation. Eggs in their cases were transferred from the opened uterus to finger bowls of sea water set in running sea water (22 to 23.5° C.).

Several methods were employed to keep embryos alive, including: 1) a slow drip of sea water into the dishes; 2) covering the dishes and changing or aerating the water several times daily; 3) Elasmobranch Ringer's solution, with or without urea, changed or aerated; 4) pasteurized sea water aerated several times daily; 5) eggs in cases were placed on wet towelling, or agar, or wet cotton in covered dishes and rinsed in sea water periodically.

Cleavage and blastula stages never appeared to develop further; older stages often lived 24 hours and many young motile embryos survived 40 to 50 hours, while a few 20 mm. embryos remained active for 3½ to 4 days. In the salt solutions, disintegration was first apparent in the yolk-sac which puckered peripherally and gradually shriveled while albumen became opaque or milky. The embryo often remained active for 10 to 24 hours after yolk-sac damage had begun. Moist chambers were somewhat better than immersion methods, particularly in gastrulation and neural fold stages which lived up to 48 hours; shrivelling of the yolk-sac and putrefaction of the yolk resulted in death.

Notes on Female Reproductive Organs

As in many elasmobranchs (Daniel, 1934), only the right ovary of *M. canis* functions in the production of ova while the vestigial left ovary is represented by a strip of tissue that may be glandular. In early June the right ovary contains approximately 12 to 24 large ova ready for ovulation (Table I).

The two oviducts unite in a common ostium situated ventrally at the anterior border of the liver. A thick-walled oviducal gland is present near the anterior end of each oviduct and fertilization presumably occurs as the egg passes through this gland which secretes albumen and the egg-case (Metten, 1939). Approximately ⅘ths of each oviduct is differentiated as a uterus opening posteriorly into the cloaca. The most noteworthy feature of the uterus in early gestation is its mucosa, a smooth, loose, highly edematous layer which can be easily stripped from the muscle coats. Thin folds of the loose mucosa extend into the lumen of the uterus and wrap around the eggs in their cases.

Eggs and Egg-Cases

In the ovary, the ovum of *M. canis* is spherical but during its passage through the oviduct and oviducal gland, it is compressed into an ellipsoid shape. Such ellipsoid ova are 30 to 38 mm. in their longest diameter and 17 to 20 mm. wide and have a volume of 2.3 to 2.8 cc. and a weight of approximately 2.9 grams in the fresh condi-

---

FIGURE 1. Semi-diagrammatic drawing in ventral view of the right oviduct and uterus of *Mustelus canis* to show orientation of egg-cases and embryos. Approximately × one-half but embryos are proportionately enlarged for the sake of clearness.

FIGURE 2. Diagrammatic transverse section of uterus (Fig. 1) to show folds of uterine mucosa inwrapping egg-case.

FIGURE 3. One egg-case of *M. canis* spread out to illustrate its dimensions and the position of the yolk. (× one-half.)

FIGURE 4. Transverse section of egg-case (Fig. 3) cut through the ovum. al, albumen; ec, egg-case; m, muscle; mu, mucosa; og, oviducal gland; os, ostium; y, yolk.

tion. The ovum is bright yellow and by the time it reaches the uterus it has been enclosed in 1 to 2 cc. of albumen and a brownish-yellow, transparent, flexible egg-case, the free ends of which are spirally twisted (Fig. 1). This twisted condition suggests that the egg was rotated during its passage through the oviduct and possibly the uterus as well. When the egg is placed in a bowl of sea water, the spiral ends of the case tend to uncoil (Fig. 5). The case is flat except where the egg is contained (Fig. 4); it tapers at either end and, completely spread out, measures 6 to 8 cm. in width and 35 to 48 cm. in length (Fig. 3).

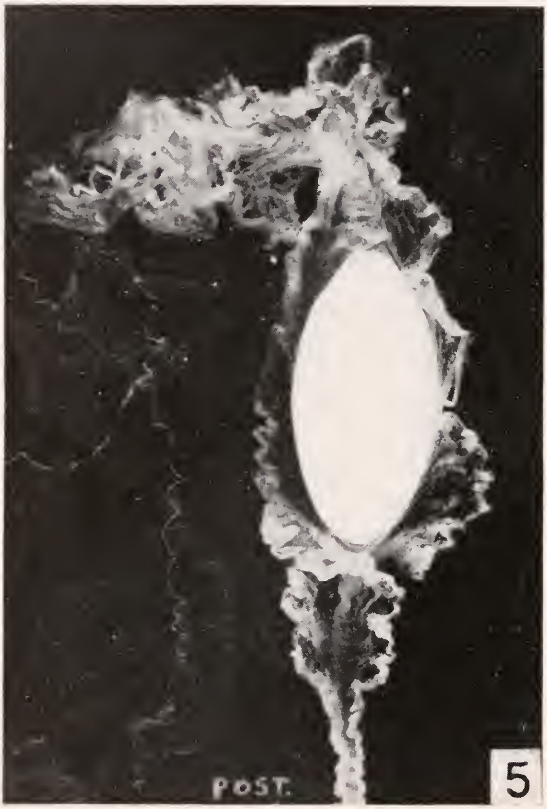


FIGURE 5. Photograph of the living ovum and egg-case of *Mustelus canis*, actual size. From a Kodachrome taken by George G. Lower. (Used by permission of Mr. Lower.)

During the first few weeks of gestation, the egg is confined to a region approximately 12 cm. from the posterior end of the case making possible an accurate determination of the orientation of an egg-case in utero. After the albumen has been absorbed, however, and the embryo is 50 to 70 mm. long (7 to 9 weeks old) the adjacent walls of the case split apart and the embryo with its yolk-sac can be made to pass freely from one end to the other showing the extent of the egg-case cavity. Whether the wall of the case remains intact during the entire gestation period can be determined only from late winter or early spring specimens, not available for the present study, but its dimensions imply such a possibility. Each egg-case is almost completely wrapped by thin folds of uterine mucosa which thus form partitions between adjacent ova as shown diagrammatically in Figure 2.

Orientation of the Blastoderm

In the earliest stages of uterine ova examined, the germinal disc is usually found at the extreme posterior tip of the egg suggesting that the egg passes into and through the oviduct with the animal pole in the advance position (Fig. 6, A). During late cleavage, however, shifting of the yolk brings the blastoderm to a less restricted region (Fig. 7, Ser. A). The characteristic orientation of the blastoderm, dorsal lip, and median axis of the embryo are illustrated in Figures 1 and 7. Typically the cephalic end of the embryo lies nearest the posterior end of the egg-case. Occasional exceptions to the typical orientation occur in which the embryo faces anteriorly or in which it lies at a right angle or oblique to the long axis of the egg. Possible explanations of such exceptions are offered in Figure 6 (B, C, D). Only early stages when the blastoderm is clearly defined and before the yolk-sac has spread extensively are reliable for determination of embryonic orientation.

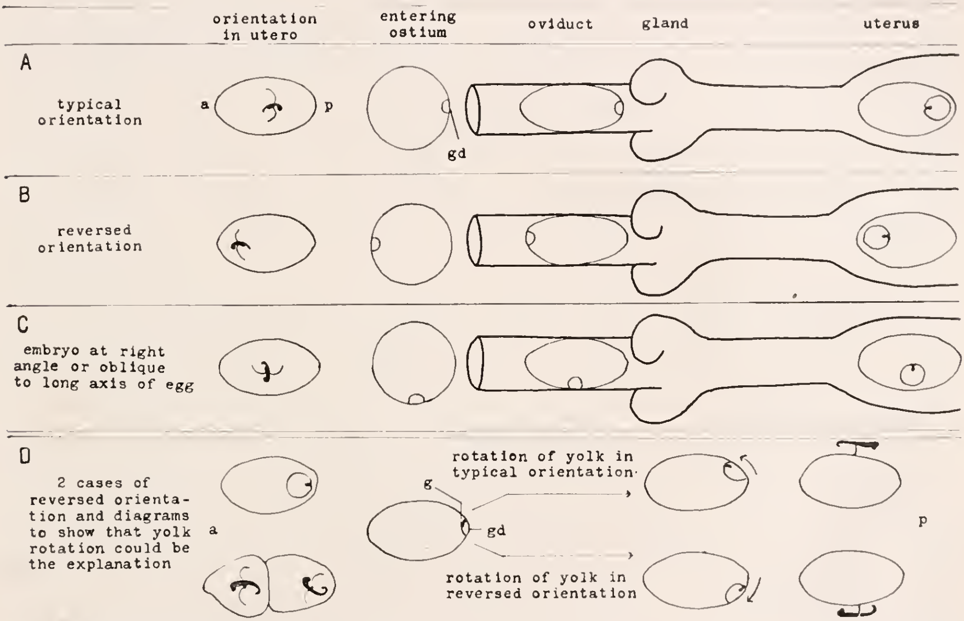

orientation in utero
entering ostium
oviduct
gland
uterus
A
typical orientation
a
p
gd
B
reversed orientation
C
embryo at right angle or oblique to long axis of egg
D
2 cases of reversed orientation and diagrams to show that yolk rotation could be the explanation
a
g
gd
rotation of yolk in typical orientation
rotation of yolk in reversed orientation
p

Figure 6. Diagrams A–C illustrate probable orientation of ovum on entering and passing through oviduct as determined from orientation of embryo observed in utero. Diagram D is an alternate explanation of reversed orientation. All ova are oriented similarly. a, anterior; g, gastrulation; gd, germinal disc; p, posterior.

Ovulation Period and Rate

Mustelus canis has a gestation period of about 10 months' duration. Pups, about 1 foot (30 cm.) in length, are born according to reports in May and pos-

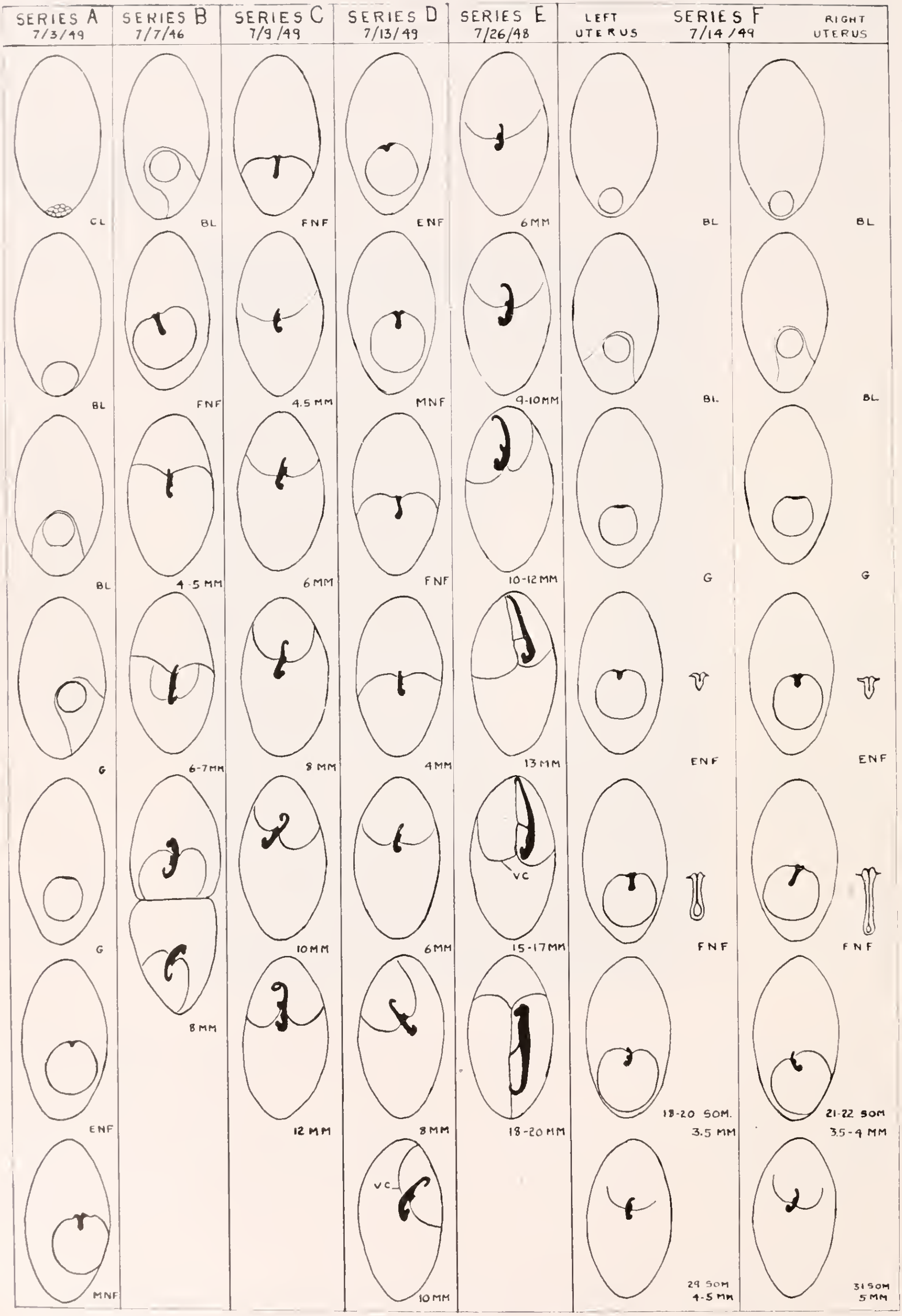

SERIES A 7/3/49
SERIES B 7/7/46
SERIES C 7/9/49
SERIES D 7/13/49
SERIES E 7/26/48
LEFT UTERUS
SERIES F 7/14/49
RIGHT UTERUS
CL
BL
G
G
ENF
MNF
BL
FNF
4-5 MM
6-7 MM
8 MM
FNF
4.5 MM
6 MM
8 MM
10 MM
12 MM
ENF
MNF
FNF
4 MM
6 MM
8 MM
VC
10 MM
6 MM
9-10 MM
10-12 MM
13 MM
VC
15-17 MM
18-20 MM
BL
BL
G
ENF
FNF
18-20 SOM.
3.5 MM
29 SOM
4-5 MM
BL
BL
G
ENF
FNF
21-22 SOM
3.5-4 MM
31 SOM
5 MM

Figure 7

sibly during late April and early June. No information is available regarding the lapse of time between birth of a "litter" and the succeeding period of ovulation but, in specimens examined in the present study, only one case of a single ovulation was observed prior to June 17 while after June 25, all mature females were pregnant. Thus an interval of 4 to 6 weeks between birth and the beginning of the next ovulation period seems a reasonable estimate.

Table I summarizes the findings in 60 specimens collected during several summers between June 8 and August 31. The number is small and the gaps which exist are recognized but, in view of the difficulty of securing material, could not be avoided. Observations, on the other hand, are consistent and are offered as representative of conditions in this species.

Data indicate that ovulation in *M. canis* usually begins in the last week of June. Specimens examined during this period contain recently ovulated eggs in cleavage and early blastoderm stages and large ova still to be extruded from the ovary. During the first two weeks of July, occasional cleavage stages are present while the oldest embryos range from neural fold stages to 10 or 12 mm. embryos. The ovary sometimes contains large ova but more frequently is totally devoid of them. After July 20, most embryos are beyond neural fold stages and in some cases measure 18 to 20 mm. in length; only one specimen containing early blastoderms was found. In August, the youngest embryos are 12 to 18 mm. and the oldest, 70 to 82 mm. in length (Table I).

From records on the condition of the ovary and embryonic stages present in utero, it may be inferred that mature females generally ovulate between late June and mid-July. Further discussion of this point will be found at the end of the section on rate of ovulation.

Calculations on the rate of ovulation are based: 1) on the series of embryonic stages in utero and 2) on the rate of development of living embryos.

1) Series of stages in utero. Figure 1 demonstrates the condition observed in *M. canis,* and said to be found in certain other viviparous elasmobranchs, that the eggs in a given uterus differ markedly in developmental stage. In *M. canis* the egg nearest the oviducal gland is always the earliest stage present and posterior to it are eggs in progressively more advanced stages arranged in sequence to the cloacal end. Figure 7 shows several examples of such series recorded in the present survey.

A second point of interest is the fact that in a given female, there is close similarity between the series of stages in the right and left uteri (Fig. 7, Series F). This condition is so characteristic that it warrants the assumption that as a rule two eggs are ovulated simultaneously or in rapid succession, one usually passing into

---

FIGURE 7. Each vertical column, A–E, represents the series of embryonic stages contained in one uterus of a specimen of *M. canis* on the date recorded. Ova are arranged in the sequence and orientation observed in utero but embryos are proportionately enlarged. The most posterior figure in Series B shows a "double-yolked" egg. Circular blastoderms in several figures are surrounded by an area lighter in color than the rest of the yolk. The periphery of the yolk-sac is not shown in older stages as it spreads rapidly and is increasingly difficult to distinguish. Early vitelline blood vessels are indicated. The two vertical columns of Series F represent embryonic stages from the left and right uteri of a single specimen to show the similarity in stage of corresponding embryos, indicating that ova are ovulated in pairs. bl. blastodisc; cl, cleavage; enf, early neural folds; fnf, fusing neural folds; g, gastrulation; mnf, mid-neural folds; vc, vitelline circulation.

each oviduct. Metten (1939) reports that oviparous dogfish commonly lay eggs in pairs and that two ova leave the ovary together. Ovulation of two eggs in *M. canis* is undoubtedly followed by a period of rest after which two more eggs are extruded and so on until all large ova have been discharged. In the oviparous *Raia brachyura,* Clark (1922)[3] noted that one egg capsule emerged from the cloaca to be followed immediately by another after which a lapse of about 24 hours intervened before the next spawning took place. Clark remarks that this is in keeping with the maturation of a single egg from each ovary. In *M. canis* both eggs are ovulated from the right ovary since the left is non-functional.

Rarely, in *M. canis,* are two eggs contained in a common egg-case and invariably such "double-yolked" eggs are in similar stages of development, clearly suggesting that they were ovulated simultaneously and possibly were held together by common tissue so that they passed into one oviduct. In one specimen (7/7/46) each uterus contained a "double-yolked" egg in the most posterior position and 4 eggs in the remainder of the series (Fig. 7, Ser. B). All four embryos of the double eggs were approximately 8 mm. in length suggesting that in this instance four eggs must have been discharged at about the same time, two entering each oviduct.

No data were collected on the migration of ova from the ovary to the ostium as none was seen during this period. Metten's (1939) interesting observations show that in *Scylliorhinus* this process is accomplished through the action of peritoneal cilia and that it is probably completed in a "matter of hours."[4]

2) *Rate of development of living embryos.* Study of the rate of development in *M. canis* was limited by the scarcity of material, its use for other purposes, and especially by the difficulty in keeping embryos alive (see Methods). Germinal disc stages and early blastoderms never appeared to develop further under laboratory conditions. Stages in which the blastoderm was thickened at its posterior margin (the beginning of the dorsal lip in gastrulation) advanced to early neural folds in approximately 35 hours at 22 to 23° C. Very early neural fold stages elongated but folds had not begun to fuse in 35 hours. Stages with well developd folds elongated and folds were fusing except at the anterior end in 35 hours. A stage with folds fused except anteriorly had formed a moving embryo with 19 to 20 pairs of somites in 12 hours (Fig. 8). In summary, an embryo would advance from the period of gastrulation to a completely fused tubular embryo in about five days.

From observations made to date, therefore, it is estimated that adjacent embryos in a given uterus in *M. canis* are at stages usually differing from each other by approximately 36 hours of developmental time. Because of the opacity of the yolk, embryos beyond late neural fold stages could not be compared in the living condition except in length. In a typical series (7/16/49) embryos measured: 3.6, 5, 6, and 8 mm. If embryos are removed from the yolk and the somites counted, the following record is typical of a series: 16–17, 29, 38, 50, 65–68, 83 somites.

Above data on the rate of development in *M. canis* are supported by the work of Vandebroek (1936) who noted in the oviparous *Scyllium canicula* (at 16° C.) that three days elapsed between the earliest sign of neural folds and the fused neural tube.

---

[3] P. 583.

[4] P. 230.

Incidental observation by the writer in 1946 showed that two eggs of the skate, *Raia erinacea,* spawned on July 13 or 14 in the aquarium of Dr. F. A. Hartmann and kindly contributed by him, were in open neural fold stages on July 21. Neural folds fused and embryos elongated from 3 to 5 mm. at the rate of about 0.5 mm. per day over a period of 3½ days and moved laterally by rhythmic contraction of somites. Unfortunately accidental injury to the egg-cases resulted in the death of the embryos.[5]

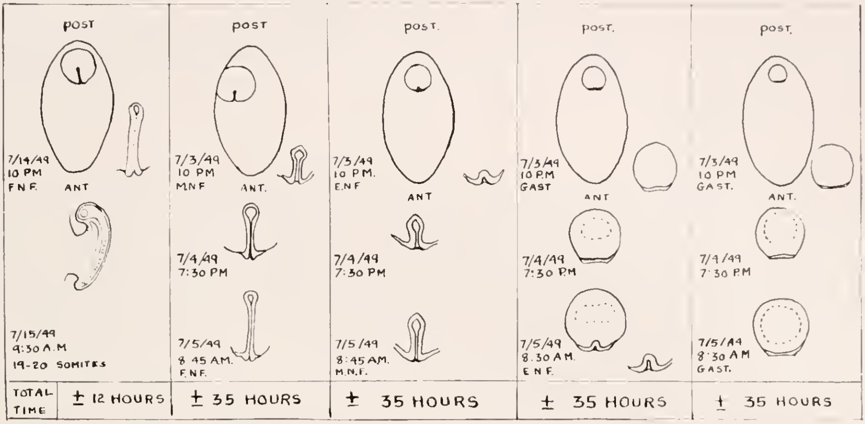

POST
7/14/49
10 PM
F.N.F.
ANT
7/15/49
9:30 A.M
19-20 SOMITES
TOTAL TIME
± 12 HOURS
POST
7/3/49
10 PM
M.N.F
ANT.
7/4/49
7:30 PM
7/5/49
8 45 AM.
F.N.F.
± 35 HOURS
POST.
7/3/49
10 P.M.
E.N.F
ANT
7/4/49
7:30 PM
7/5/49
8:45 AM.
M.N.F.
± 35 HOURS
POST.
7/3/49
10 P.M
GAST
ANT
7/4/49
7:30 P.M
7/5/49
8.30 A.M.
E.N.F.
± 35 HOURS
POST.
7/3/49
10 PM
GAST.
ANT.
7/4/49
7·30 P.M
7/5/A4
8·30 A M
GAST.
± 35 HOURS

FIGURE 8. Rate of development of living embryos of *M. canis.* Labels indicate the orientation of ova in utero. Eggs in egg-cases were placed on wet cotton in covered finger bowls set in running sea water at 22–23° C.

Clark (1926), at the Plymouth Laboratory, opened egg capsules of *R. brachyura* tagged at the time of spawning and found cleavage stages through the fourth day of incubation. His next report is that of an early neural fold stage, aged 24 days, while embryos of 4 to 4.5 mm. were observed after 35 and 43 days. Clark's series is incomplete with no data for embryos between 7.5 and 58.5 mm. nor did he follow the development of individual eggs. The exceedingly slow rate in *R. brachyura* as compared with that of other elasmobranchs mentioned above may be a species difference and may be attributed in part to the low temperature of the laboratory tanks at Plymouth (13.4–16° C.).

On the basis of the above data on the development of individual eggs of *M. canis,* together with the marked difference in stage and arrangement in sequence of the embryos in a uterine series, it can be estimated that successive ovulations usually of two eggs, occur in this species about every 30 to 40 hours. This is a reasonable estimate in view of Clark's (1922) observation of a 24 hour interval between spawnings in *R. brachyura* but it is an approximation only. More data on living embryos are necessary before a definite statement can be made. Assuming, however, that *M. canis* ovulates about every 36 hours, it can be calculated that in the fish represented by Series A (Fig. 7, 7/3/49), the first ovulation occurred about June 22 and the last about July 1; in Series C (7/9/49), the first ovulation took place about June 20, the last about June 27; Series F (7/14/49), first, June 29; last,

---

[5] The capsule of the skate egg may be shaved thin over the embryo to facilitate observation.

July 9. In Series B (7/7/46) the great difference between the two youngest embryos suggests an interval of about 5 days between ovulations, an instance of irregularity in the cycle. From records of a number of series of embryos (Table I) it is concluded that ovulation in *M. canis* usually begins between June 20 and July 7 and is terminated between June 25 and July 20.

An ovulatory period limited to a few weeks in the summer is in distinct contrast to the situation in oviparous elasmobranchs, many of which have been found to breed throughout the year (Clark, 1922; Metten, 1939). In species with uterogestation, however, it is not surprising that ovulation is confined to a short period between pregnancies. That such is also the case in *Squalus acanthias* is known from the work of Hisaw and Albert (1947) who report that ovulation occurs every two years following a gestation of 20 to 22 months.

Egg-cases without yolk. Occasionally empty egg-cases are found in the uterus of *M. canis*. In one instance (8/4/46) a fish opened soon after collecting contained 5 eggs in cases in the right uterus and 5 cases with albumen but no sign of yolk in the left uterus. There were no crushed or disintegrating ova. Embryos in the right uterus measured 15 to 28 mm. in length and were in excellent condition. A possible explanation of this phenomenon is that the lumen of the left oviduct had been occluded (a point unfortunately not determined) so that all ova entered the right oviduct.[6] Since 5 empty cases were present in the left uterus, however, the conclusion seems warranted that at each ovulation of an egg, or eggs, secretion of albumen and egg-case by both right and left oviducal glands occurs. If only one egg is ovulated, one of the two cases contains no yolk.

This assumption is supported by two instances in which, when the uteri of a specimen contained an unequal number of ova, an empty egg-case was found in the uterus with the smaller number in a position corresponding to that of the last egg ovulated on the opposite side (7/3/49; 7/7/49). In some specimens where unequal numbers of eggs were found, it was probable that one or more eggs had been extruded from a uterus, but in the two examples given above, the stages of embryos present, the position of the empty egg-cases at the most anterior end of the uterus, and the absence of any trace of yolk eliminated such an explanation.[7]

In a few instances skate egg-capsules spawned in Dr. Hartmann's aquarium contained no yolk. Clark (1922) in his report on rays and skates, noted "a small percentage of perfectly formed, undamaged egg-capsules contained some albumen but no yolk."[8] He suggested that such capsules had been closed before eggs reached them but it seems entirely possible that such yolkless capsules may be other examples in which both oviducal glands secreted egg-cases although only one egg was ovulated.

Metten (1939) presents conclusive evidence in *Scylliorhinus* that the ovum does not act as a direct stimulus for the secretion of the egg-case, in showing that over one-half of the elaborate case of this species is formed before the egg enters the oviducal gland. He cited one instance also of a fully formed egg-case lacking the yolk.

---

[6] When this specimen was obtained, the probability of the ovulation of two eggs at one time was unknown to the author so that the size of each of the five embryos was not recorded. It is possible that there were pairs of embryos of the same size, but the range of 15 to 28 mm. suggests the unusual occurrence of five single ovulations at spaced intervals.

[7] In 5 specimens with unequal numbers of ova (Table I), no data were recorded on the presence or absence of empty egg-cases because at the time the writer was concerned chiefly with securing embryos for another study and did not realize the importance of such evidence.

[8] P. 580.

Although experimental work is needed for definite proof, it is highly probable that oviducal glands in elasmobranchs, as in other vertebrates, are controlled by endocrine secretions. Wallace (1903) noted corpora luteal tissue in ruptured ovarian follicles of *Spinax*, and Hisaw and Albert (1947) have reported the differentiation of luteal tissue after ovulation in *Squalus acanthias*. It is not unlikely, therefore, that ovarian hormones, present at the time of ovulation or slightly preceding it, stimulate the secretion of a single egg-case by each oviducal gland irrespective of the number of ova discharged. This assumption would explain the occurrence of empty egg-capsules as well as that of "double-yolked" eggs.

SUMMARY

1. The ovum and egg-case of *Mustelus canis,* a viviparous elasmobranch, are described and the orientation of the egg-case in utero is noted.

2. The blastoderm is typically located near the cloacal end of the yolk and the dorsal lip of the blastopore forms at the anterior border of the blastoderm when the egg is oriented as it lies in utero. Occasional cases of reversed and oblique orientation are found.

3. In early June, mature females are post-partum. The functional right ovary contains large ova soon to be ovulated while the left ovary is vestigial.

4. The ovulation period extends from about June 20 to July 20. Four to eight ova are generally found in each uterus of a pregnant female and, from a comparison of stages present, it is probable that as a rule two ova are discharged simultaneously with an interval tentatively estimated at 30 to 40 hours between most subsequent ovulations.

5. Ova are arranged in sequence in each uterus with the most advanced embryo nearest the cloacal end.

6. Stages of embryos present in utero between June 23 and Aug. 31 are tabulated. An egg ovulated at the beginning of this period would develop into an embryo with a maximum length of about 80 mm. in 9 weeks time.

7. From observations made on living material, blastoderms with thickened dorsal lips (gastrulation stages) are estimated as approximately 5 days younger than fused neural tube stages 3.5 to 4 mm. in length.

8. On the basis of egg-cases devoid of yolk as well as from the occurrence of "double-yolked" eggs, it is suggested that oviducal glands are controlled by ovarian hormones and that a single egg-case is secreted simultaneously by both right and left glands irrespective of the number of ova discharged at a given ovulation.

LITERATURE CITED

BIGELOW, H. B. AND W. W. WELCH, 1924. Fishes of the Gulf of Maine. *Bull. U. S. Bureau of Fisheries,* **40**: 44–50.

CLARK, R. S., 1922. Rays and skates (Raiae). No. 1, Egg capsules and young. *Jour. Marine Biol. Assoc. United Kingdom,* New Series, **12**: 577–643.

CLARK, R. S., 1926. Rays and skates. No. 2, Description of embryos. *Jour. Marine Biol. Assoc. United Kingdom,* New Series, **14**: 661–683.

FORD, E., 1921. A contribution to our knowledge of the life-histories of the dogfishes landed at Plymouth. *Jour. Marine Biol. Assoc. United Kingdom,* New Series, **12**: 468–505.

DANIEL, J. F., 1934. The elasmobranch fishes. Berkeley, California.

HISAW, F. L. AND A. ALBERT, 1947. Observations on the reproduction of the spiny dogfish, *Squalus acanthias*. *Biol. Bull.*, **92**: 187–199.

HUBBS, C. L., 1938. Scientific names of the American "smooth dogfish." *Mustelus canis* (Mitchell), and of related European species. *Occ. papers, Mus. Zool. U. of Mich.*, **14**: No. 374.

METTEN, H., 1939. Studies on the reproduction of the dogfish. *Phil. Trans. Roy. Soc. London*, **230**: 217–238.

RANZI, S., 1932. Le basi fisio-morfologiche dello sviluppo embrionale dei Selaci. Parte I. *Pubbl. d. Staz. Zool. Napoli,* **12**: 209–290.

RANZI, S., 1934. Le basi fisio-morfologiche dello sviluppo embrionale dei Selaci. Parti II & III. *Pubbl. d. Staz. Zool. Napoli,* **13**: 331–437.

SCAMMON, R. E., 1911. Normal plates of the development of *Squalus acanthias*. *Normentafeln z. Entwgesch. d. Wirbeltiere,* **12**: 1–140.

SMITH, B. G., 1942. The heterodontid sharks: their natural history, and the external development of *Heterodontus japonicus* based on notes and drawings of Bashford Dean. *Amer. Mus. Nat. Hist:* The Bashford Dean Memorial Volume Archaic Fishes, Article 8: 649–770.

TEN CATE-HOEDEMAKER, N. J., 1933. Beitrage zur Kenntnis Plazentation bei Haien und Reptilien. Der Bau der reifen Plazenta von *Mustelus laevis* Risso und *Seps chalcides* Merr. *Zeitschr. f. Zellforsch. u. mik. Anat.*, **18**: 299–345.

TEWINKEL, L. E., 1943. Observations on later phases of embryonic nutrition in *Squalus acanthias*. *Jour. Morph.*, **73**: 177–205.

VANDEBROEK, G., 1936. Les mouvements morphogénétique au cours de la gastrulation chez *Scyllium canicula* Cuv. *Arch. de Biol.,* **47**: 499–584.

WALLACE, W., 1903. Observations on ovarian ova and follicles in certain teleostean and elasmobranch fishes. *Quart. Jour. Mic. Sci.*, n.s., **47**: 161–213.

WHITE, E. G., 1937. Interrelationships of the elasmobranchs with a key to the order Galea. *Bull. Amer. Mus. Nat. Hist.*, **74**: 25–138.

THE COMPARATIVE EFFECTS OF TELEOST AND BEEF PITUITARY ON CHROMATOPHORES OF COLD-BLOODED VERTEBRATES[1]

GEORGE F. WEISEL

Montana State University, Missoula

Treatment of most cold-blooded vertebrates, including catfish and eels, with pituitary gland induces a distal migration of pigment in their melanophores (Parker, 1948). However, there is evidence that the melanophores of some teleosts, such as killifish and Phoxinus, react oppositely to this treatment. In these fish the melanin concentrates (Hewer, 1926; Matthews, 1933). This variance with teleosts may be due to physiologically distinct chromatophores or it may be caused by differences in the source or preparation of the pituitary used. For instance, commercial preparations often contain such preservatives as chloretone which also effect color changes and therefore may lead to false interpretation.

An effort is made here to compare the effects of pituitary extracts from two widely separated groups of vertebrates, teleosts and mammals, on the chromatophores of elasmobranchs, teleosts, amphibians and reptiles to determine if there are differences in either the extracts or the target organs. Particular emphasis is placed on the chromatophores of the anomolous bony fishes.

Materials and Methods

The following animals were utilized for this study: the elasmobranch *Urobatis halleri* (round stingray); the teleosts *Ameiurus melas* (black bullhead), *Fundulus parvipinnis* (California killifish), *Gambusia affinis* (mosquito fish), *Leuresthes tenuis* (grunion), *Girella nigricans* (opaleye), *Lepomis cyanellus* (green sunfish), and *Gillichthys mirabilis* (mudsucker); the amphibians *Hyla regilla* (Pacific tree frog) and *Rana pipiens* (meadow frog); and the reptile *Anolis carolinensis* (Carolina anole). After being adapted to white and black backgrounds, these animals were treated with extracted whole pituitary from fish and beef. Fundulus and Girella were also injected with Parke, Davis and Co. obstetrical pituitrin and beef posterior lobe extracts. Controls, similarly adapted, were injected with fish brain or with saline solution, or remained untreated. The controls always had the same light and temperature as the test animals.

The fish pituitaries were from daily landings of sheepshead (*Pimelometopon pulchrum*), barracuda (*Sphyraena argentea*), yellowfin tuna (*Neothunnus macropterus*), and white sea bass (*Cynoscion nobilis*). The pituitaries from different species were not mixed, although no differences were observed in their effects. Most of the experiments and all the final tests were performed with barracuda preparations. The whole gland was used since it was found impractical to separate the

---

[1] This investigation was aided by a grant from the Research Committee, Montana State University. Most of the work was done at Scripps Institution of Oceanography, La Jolla, California and includes part of the material in a thesis submitted to the University of California at Los Angeles in partial fulfillment of the requirements for the Doctor of Philosophy degree.

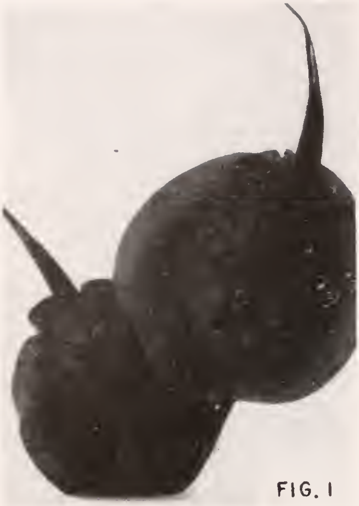


FIG. 1

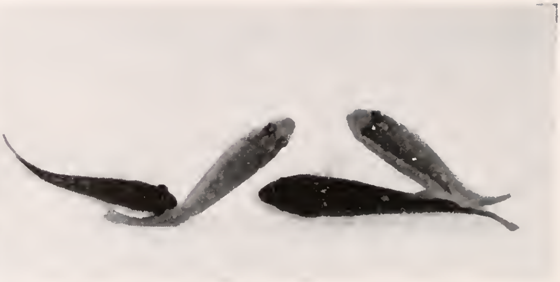


FIG. 2

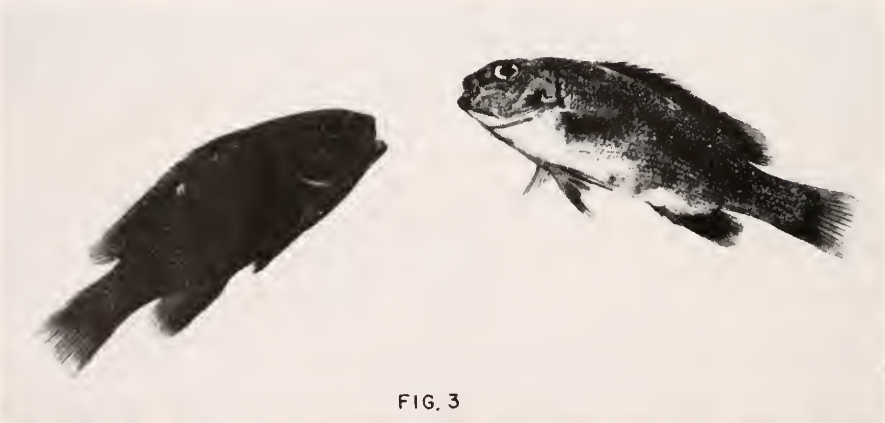


FIG. 3

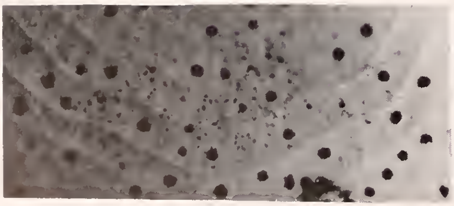


FIG. 4

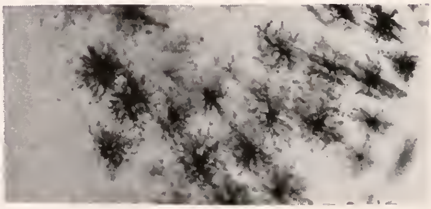


FIG. 5

FIGURE 1. Effect of fish pituitary on the coloration of *Urobatis halleri* kept on a white background. The dark ray on the left had been injected with fish pituitary and the pale ray on the right with fish brain. Photograph of living specimens.

FIGURE 2. Effect of fish and beef pituitary on the coloration of *Fundulus parvipinnis* kept on a black background. The two pale fish had been treated with fish pituitary and the two dark ones with beef pituitary. Photograph of live fish.

small, united lobes. The glands were placed in acetone and finely ground in a mortar. The powder was extracted five or six times with acetone, air dried, and put in glass ampoules which were sealed under reduced pressure. Over 1000 fish pituitaries were processed in this manner. One barracuda pituitary was equivalent to 4.2 mg. of the desiccated material.

Beef pituitaries were treated in the same fashion as the fish glands. Extracts were made of both the whole gland and the posterior lobe of freshly killed animals.

Injections were made intraperitoneally or, in the case of adult frogs, in the dorsal lymph sac. The dosages used in each test are recorded in the results.

RESULTS

Urobatis halleri

Black-adapted and white-adapted rays were each injected with 7 mg. fish pituitary. Similarly adapted rays, serving as controls, were injected with 7 mg. fish brain. They were replaced in their respective white and black containers. After 2 hours those which had been treated with pituitary and were on a white background were slightly darker and had more distinct mottlings than those on the same background which had been treated with brain (Fig. 1). The darkening effect of the pituitary endured for 18 hours. No differences could be detected in the coloration of rays in black containers. Twenty-four hours after their treatment with brain, the controls were injected with fish pituitary. They darkened.

Ameiurus melas

Bullheads placed in dimly lighted aquaria for 48 hours paled to yellow ocher over the entire body, except on the tips of the barbels and fins. They became black when injected with either 5 mg. barracuda pituitary, 5 mg. whole beef pituitary or 0.2 cc. pituitrin, but remained pale when treated with fish brain.

Fundulus parvipinnis

Due to an obvious difference in coloration between mature males and females, only healthy female killifish were utilized.

Ten white-adapted and 10 black-adapted animals treated with 2 mg. fish pituitary became pale within 20 minutes (Fig. 2). They became slightly darker after 6 hours, but whether on a white or black background, they remained noticeably paler than any of the controls for 72 hours.

Beef whole gland was administered in a larger dose (3 mg.) than was the fish extract, yet it caused no change in the coloration of white-adapted or black-adapted fish (Fig. 2). The same was true for the fish treated with extracts of the posterior

---

FIGURE 3. Effect of fish pituitary and pituitrin on the coloration of *Girella nigricans* kept on a black background. The pale fish on the right had been treated with pituitary and the dark one on the left with pituitrin. Notice the pale iris of the fish injected with the fish extract. Photograph of live fish.

FIGURE 4. Photomicrograph of a portion of a scale from Fundulus injected with fish pituitary. The melanin is punctate.

FIGURE 5. Photomicrograph of a portion of a scale from Fundulus injected with beef pituitary. The melanin is diffuse. Untreated controls appeared the same.

lobe of beef or with pituitrin. Controls injected with Ringer's solution or fish brain also exhibited no color change.

As many as 6 fresh Fundulus glands, implanted in the body cavity, did not pale black-adapted killifish. However, when a greater quantity of fresh fish material (2 glands of Sebastodes) was introduced, enough of the substance was present to cause them to blanch to a light yellow, even though they were on a background conducive for darkening. Killifish implanted with a comparable amount of fresh fish brain adapted to their black background.

The color of the variously treated fish was correlated with the microscopic appearance of the chromatophores (Table I). Fish pituitary, like adrenalin, caused a proximal migration of melain, whereas barracuda brain and mammalian extracts had no apparent effect on black- or white-adapted animals (Figs. 4 and 5).

TABLE I

Microscopic appearance of melanophores on scales of Fundulus parvipinnis

Material injected	Total number melanophores counted*	% Punctate	% Intermediate	% Diffuse
Barracuda brain	1211	3	16	81
Beef whole pituitary	894	9	22	69
Beef posterior lobe	1055	6	7	87
Obstetrical pituitrin	1107	2	13	85
Desiccated fish pituitary	1291	76	19	5
Fresh fish pituitary	988	68	19	13
Adrenalin chloride	1402	88	11	1

* Counts were made on scales from two fish for each material injected. Each circulus of a scale was taken in turn and followed around.

Punctate = melanin concentrated in a small spot in the center of the melanophore; Intermediate = stellate, melanin only partially dispersed; Diffuse = melanin well dispersed in the radiating processes of the melanophores.

The xanthophores of *F. parvipinnis* are much smaller than the melanophores and their radiating processes are difficult to discern. Pale fish, which had been treated with fish pituitary, showed most of the xanthophores expanded. In the dark fish the xanthophores were largely obscured by the radiating processes of the overlying melanophores. Accurate count of expanded or contracted xanthophores could not be made.

The fish extracts were also tested on Fundulus with denervated dark bands on their caudal fin. The method used in denervation was essentially the same as that employed by Parker (1934). A cut was made across two caudal fin rays just posterior to the basal blood vessel arc of the tail. Within a few hours a dark band of expanded melanophores was formed distal to the cut which, according to Parker, results from dispersing nerves stimulated by injury. Ten specimens with such denervated bands were injected with fish pituitary. The dark denervated bands paled like the rest of the body.

Girella nigiricans

Ten olivaceous black opaleyes, injected with sufficient fresh fish pituitary or with 3 to 4 mg. fish pituitary extract, commenced to blanch in 10 to 30 minutes and remained pale for 30 hours. The normally blue iris became gold for the first few hours after injection (Fig. 3).

Barracuda brain, pituitrin and extracts of beef whole gland or posterior lobe had no apparent action.

Any disturbance caused light-colored opaleyes, whether pale due to sojourn on a white background or from injections of teleost pituitary, to darken when handled or otherwise disturbed. Some microscopic analysis of color change, however, was possible throught a study of their scales. The extreme pallor of those injected with the teleost pituitary was confirmed as being due to concentration of melanin.

The outlines of the xanthophores of Girella are more clearly defined than are those of Fundulus. Eight-hundred to 1200 of these chromatophores were counted on scales from each fish. Xanthophores of fish treated with fish pituitary averaged 93 per cent expanded and 7 per cent intermediate, whereas for fish treated with mammalian preparations or fish brain they ranged from 8–28 per cent expanded. The majority were punctate.

Gambusia affinis, Leuresthes tenuis, Lepomis cyanellus, Gillichthys mirabilis

Mosquito fish, grunion, sunfish and mudsuckers all paled and remained so for at least 48 hours after treatment with fish pituitary. At least 10 fish of each species were tested. The mosquito fish became so transparent on the head that the outlines of the brain showed, indicating that the perineural as well as the skin melanophores had contracted. None of these species showed a visible reaction to fish brain or to mammalian preparations.

Hyla regilla

Six white-adapted frogs became practically black one hour after having 1 to 3 mg. fish pituitary injected into their dorsal lymph sac. Those which received the larger doses did not darken more quickly than those which received smaller amounts. However, there was a correlation between the dosage and the duration of melanophor expansion. Frogs injected with 3 mg. remained dark for approximately 96 hours, whereas those which received 1 mg. returned to the light phase in 18 hours. The effects of a 2 mg. dose lasted 24 hours.

Six controls treated with fish brain exhibited no color change.

Rana pipiens

Twenty white-adapted meadow frogs injected with either fish or beef pituitary darkened within an hour. Even the gold in the iris was supplanted by black. Some commenced to pale by 24 hours, and it was noticed at this time that the iris had largely regained its gold. Sections of frog skin in saline suspensions of beef or fish pituitary also darkened.

No differences were noticed between the action of the fish and beef preparations. No attempts were made to assay the materials, but when used in the same concentrations (6 mg.), the fish and beef extracts induced darkening at approximately the same time and the effects wore off at about the same time.

There were no observable color changes in any of the various controls nor in black-adapted frogs injected with pituitary preparations.

Tadpoles of Rana pipiens

Tadpoles which received either fish or beef pituitary and were then placed in white porcelain bowls became considerably darker than controls. Microscopic examination of the tails of these tadpoles revealed that the majority of the melanophores had diffuse melanin and that most of the leucophores had concentrated pigment (Table II). Many of the dermal melanophores formed a blotchy reticulum involving several of these black chromatophores. On the other hand, most of the melanin was concentrated and the guanine of the leucophores was dispersed in the tails of controls; a condition just reverse to that in animals treated with pituitary.

Table II

*Microscopic appearance of chromatophores on tails of Rana pipiens larvae**

Material injected	Melanophores			Leucophores		
	Punctate	Intermediate	Diffuse	Punctate	Intermediate	Diffuse
Barracuda brain	70	27	3	31	69	0
Ringer's	76	18	6	20	60	20
Barracuda pituitary	20	10	70	95	5	0
Beef pituitary	23	18	59	88	12	0

* Counts were made on two individuals for each different material injected. A total of 500 chromatophores of each type was counted in each individual. The counts are expressed as percentages.

Anolis carolinensis

It was found best to make observations on the lizard at night because, although the dark-adapted lizards were kept in battery jars painted black and covered to exclude light, they were more uniformly green at night than during daytime. Anolis exhibits a diurnal melanophore rhythm, which is attributable to the pituitary (Rahn and Rosendale, 1941).

Fifteen green, dark-adapted lizards injected with 4 mg. barracuda pituitary commenced to darken at the point of injection in approximately 1 minute. Twelve treated with the same amount of beef whole gland reacted similarly. In an hour their backs were black to chestnut brown, which color they retained, night as well as day, for 30 hours. Another group of 15 lizards which received 4 mg. barracuda brain or which were untreated remained green throughout the night, darkened slightly the next day, and were green again on the following night.

Compared with their controls, brown lizards from lighted jars showed the effects of the pituitary only at night. During the day the controls were about as dark as the experimentals.

Sections of skin from the backs of green lizards, placed in suspensions of fish or beef pituitary and Ringer's solution, also proved that both extracts had a darkening action.

Discussion

Investigators agree that hypophysectomy causes elasmobranchs to blanch and, conversely, that they darken when injected with pituitary of their own species or with the mammalian gland (Lundstrom and Bard, 1932; Hogben, 1936; Parker, 1936). My experiments indicate further the non-specific darkening effect of pituitary inasmuch as rays (*Urobatis halleri*) were shown to darken slightly when treated with fish pituitary.

Unlike the elasmobranchs, different species of bony fishes exhibit diverse reactions to the chromatic principle (or principles) of the pituitary. Removal of the pituitary of Ameiurus causes the melanin to concentrate (Osborn, 1941; Parker, 1941), but a similar operation on Fundulus apparently has little or no effect on the state of the melanophores (Matthews, 1933; Fries, 1943). Injections of either teleost, amphibian or mammalian pituitary causes Ameiurus to darken (Odiorne, 1933; Parker, 1941). On the other hand, Odiorne was unable to show any melanophore response with pituitrin treated Fundulus, and Matthews (1933) observed that the melanophores on isolated scales of the same species contracted and the xanthophores expanded when they were treated with fish pituitary.

My results with Fundulus injected with fish pituitary agree most closely with Matthews' work as cited above and with Hewer's (1926) observations that cod pituitary contracts melanophores but expands xanthophores of Phoxinus. The paling of my killifish was so striking that there could be no mistaking the reaction, and the contracted condition of the melanophores was fully verified by microscopic examination. Gambusia, Gillichthys, Lepomis, Leuresthes and Girella all reacted similarly to Fundulus. It is very probable, however, that other species of fish may react like catfish and eels. Certainly no generalized rule can now be made for the color changes that teleosts as a group undergo when treated with pituitary preparations.

The literature on the subject of teleost response to mammalian preparations likewise indicates that all fish do not respond in a similar manner. However, a wide variety of hormone preparations have been used, and it is doubtful if any two contained similar pituitary substances in equal concentration. Using Fundulus alone as an example, commercial posterior lobe extracts causes melanophores to contract (Wyman, 1924); obstetrical pituitrin has no effect except on denervated areas where it expands melanophores (Kleinholz, 1935); and pituitrin has no action on melanophores but antuitrin contracts them (Odiorne, 1933). Although the mammalian extracts used in this study obviously darkened Ameiurus, they had no visible effect on the other bony fishes tested.

With one exception, melanophores of amphibians expand when the animals are treated with teleost, amphibian, reptilian or mammalian pituitary (Allen, 1920; Kleinholz, 1935; Gray and Ford, 1940). The exception is Diemyctylus, which reacts oppositely by paling when injected with pituitrin (Collins and Adolph, 1926). My results with amphibians were like those of previous investigators in indicating no specificity of chromatophorotropic hormone.

Falling in with the main line of cold-blooded vertebrate evolution, the reptiles are also uniform in their general response. Hypophysectomy leads to a permanent pallor (Parker, 1938; Rahn, 1941). Injection of fish, amphibian, reptilian, avian, or mammalian pituitary darkens them (Kleinholz, 1938, 1940; Parker, 1938; Rahn, 1941). In this study, the lizards behaved as expected from reports by previous investigators. Both fish and beef pituitary darkened green Anolis. There did not appear to be any qualitative difference in reaction to fish or beef preparations.

It would appear, therefore, that melanophores of nearly all cold-blooded vertebrates except the bony fishes react similarly to the chromatophorotropic principle. Also, pituitaries from all classes of vertebrates apparently have a like effect on all but the teleosts. The diverse chromatic reaction of teleosts seems to have a phylogenetic significance. Species, such as Ameiurus and Anguilla, have melanophores which react like those of other cold-blooded vertebrates. Species belonging to the higher orders, which have diverged more from the common trunk of vertebrate evolution, such as Fundulus and Girella, have melanophores which react oppositely to fish pituitary and exhibit little or no reaction to mammalian preparations.

There is at present no reason to believe that melanophores in different species cannot react oppositely to the same stimulus. It has been shown that potassium chloride will expand amphibian melanophores (Thörner, 1929) but contract those of certain fish (Spaeth, 1913). Also, different types of chromatophores in an individual animal may react differently to the same stimulus as was demonstrated in this investigation with tadpoles, Fundulus and Girella and by Allen (1925), Hewer (1926) and Matthews (1933).

Not only is there a differential reaction of melanophores in different species, as well as of different chromatophore types in an individual, but pituitary extracts from different sources are seen to be unlike in their chromatophorotropic activity when tested on higher bony fishes. The chromatic changes induced in Fundulus and Girella by fish extracts were undeniably different from their negative response to mammalian extracts. The mammalian material contained a melanophore expanding principle which was effective on catfish, frogs and lizards but which was ineffective on chromatophores of advanced fish, presumably because of low sensitivity or nonsensitivity of the fish target organs to this substance. The teleost extracts have a principle which, although like the mammalian, is capable of darkening rays, catfish, frogs and lizards, and differs in that it contracts melanophores of higher teleosts.

It is possbile that there are two chromatophorotropic factors in the fish pituitary. This appears to be the case for amphibians (Hogben and Slome, 1936; Steggerda and Soderwall, 1939). If there are two factors in the fish pituitary, then the melanophores of higher fish must be more sensitive to the contracting one than to the expanding one, whereas the melanophores of elasmobranchs, etc. must be most sensitive to the expanding principle.

It has not been the purpose of this investigation to determine whether the pituitary plays a normal role in the color changes of fish. The dosages used were purposely held large in order to elicit responses that would be definite. Also, the animals were placed in an environment which would normally effect color changes opposite to that wrought by pituitary treatments, in order to make more evident the comparisons with controls. Large doses were required to overcome the opposition factors.

SUMMARY

1. Fish and beef acetone-desiccated pituitaries cause a distal migration of pigment granules in melanophores when injected into the elasmobranch *Urobatis halleri*, the teleost *Ameiurus melas*, the amphibians *Hyla regilla* and *Rana pipiens*, and the reptile *Anolis carolinensis*. These preparations not only disperse melanin but also

induce a proximal migration of the white granules in leucophores of *Rana pipiens* larvae.

2. Fish pituitary injected into the teleosts *Gambusia affinis, Fundulus parvipinnis, Girella nigricans, Gillichthys mirabilis, Lepomis cyanellus* and *Leuresthes tenuis* causes a proximal migration of melanin and, at least in the first three species, a distal migration of pigment in the xanthophores. Desiccated fish brain, beef whole gland, beef posterior lobe and commercial pituitrin have no obvious effect on their chromatophores.

3. There is an opposite migration of melanin in certain different species of teleosts treated with fish pituitary. The diverse chromatic reaction of teleosts may have phylogenetic significance. The more primitive teleosts appear to react like other classes of cold-blooded vertebrates, whereas the higher teleosts, which have diverged more from the common line of vertebrate evolution, do not.

4. There is a differential in reaction of different types of chromatophores in a single species to treatment with the same pituitary preparations.

5. Pituitary material from different sources may have unlike chromatophorotropic action. Beef and fish pituitary have a similar action (expansion) on melanophores of Urobatis, Ameiurus, Rana and Anolis. However, on such teleosts as Fundulus and Girella, fish extracts have the opposite effect and mammalian extracts have no apparent effect.

Acknowledgments

I wish to thank Dr. Carl L. Hubbs for his enthusiasm and inspiration during the course of this work, Dr. Boyd Walker for the time he kindly donated to help in dissecting out fish pituitaries, and the members of Bregante's Fish Co. in San Diego, California, for their cheerful cooperation which made even the slicing of numerous fish heads enjoyable.

Literature Cited

Allen, B. M., 1920. Experiments in the transplantation of the hypophysis of adult *Rana pipiens* to tadpoles. *Science,* **52**: 274–276.

Allen, B. M., 1925. Color change induced in Rana larvae by implantation of the intermediate lobe of the hypophysis. *Anat. Rec.,* **31**: 302–303.

Collins, H. H. and E. F. Adolph, 1926. The regulation of skin pattern in an amphibian, Diemyctylus. *Jour. Morph.,* **42**: 473–522.

Fries, E. F. B., 1943. Pituitary and nervous control of pigmentary effectors, especially xanthophores, in killifish (Fundulus). *Physiol. Zool.,* **16**: 199–212.

Gray, S. W. and W. Ford, 1940. The effect of the crustacean eye-stalk hormone upon water metabolism and melanophore expansion in the frog. *Endocrinol.,* **26**: 160–162.

Hewer, H. R., 1926. Studies in colour changes of fish. Part I. The action of certain endocrine secretions in the minnow. *Jour. Exp. Biol.,* **3**: 123–140.

Hogben, L., 1936. The pigmentary effector system. VII. The chromatic function in elasmobranch fishes. *Proc. Roy. Soc. London, Ser. B,* **120**: 142–158.

Hogben, L. and D. Slome, 1936. The pigmentary effector system. VIII. The dual receptive mechanism of the amphibian background response. *Proc. Roy. Soc. London, Ser. B,* **120**: 158–173.

Kleinholz, L. H., 1935. The melanophore-dispersing principle in the hypophysis of *Fundulus heteroclitus. Biol. Bull.,* **69**: 379–390.

Kleinholz, L. H., 1938. Studies in reptilian colour changes. II. The pituitary and adrenal glands in the regulation of the melanophores of *Anolis carolinensis. Jour. Exp. Biol.,* **15**: 474–491.

Kleinholz, L. H., 1940. The distribution of intermedin: First appearance of the hormone in the early ontogeny of *Rana pipiens. Biol. Bull.,* **79**: 432–438.

Lundstrom, H. M. and P. Bard, 1932. Hypophysial control of cutaneous pigmentation in an elasmobranch fish. *Biol. Bull.*, **62**: 1–9.

Matthews, S. A., 1933. Color changes in Fundulus after hypophysectomy. *Biol. Bull.*, **64**: 315–320.

Odiorne, J. M., 1933. The effects of the pituitary hormones on the melanophores of fishes. *Proc. Nat. Acad. Sci. Wash.*, **19**: 745–749.

Osborn, C. M., 1941. The effect of hypophysectomy upon the growth of melanophores in the catfish (*Amciurus melas*). *Anat. Rec.*, **81**: 59–60.

Parker, G. H., 1934. Neurohumors as activating agents for fish melanophores. *Proc. Amer. Phil. Soc.*, **74**: 177–184.

Parker, G. H., 1936. The reactions of chromatophores as evidence for neurohumors. *Cold Spring Harbor Symposia Quant. Biol.*, **4**: 358–370.

Parker, G. H., 1938. The colour changes in lizards, particularly in Phrynosoma. *Jour. Exp. Zool.*, **15**: 48–73.

Parker, G. H., 1941. The method of activation of melanophores and the limitations of melanophore responses in the catfish Ameiurus. *Proc. Amer. Phil. Soc.*, **85**: 18–24.

Parker, G. H., 1948. Animal colour changes and their neurohumors. A survey of investigations 1910–1943. *Cambridge University Press,* England.

Rahn, H., 1941. The pituitary regulation of melanophores in the rattlesnake. *Biol. Bull.*, **80**: 228–237.

Rahn, H. and F. Rosendale, 1941. Diurnal rhythm of melanophore hormone secretion in Anolis pituitary. *Proc. Soc. Exp. Biol., N. Y.*, **48**: 100–102.

Spaeth, R. A., 1913. The physiology of the chromatophores of fishes. *Jour. Exp. Zool.*, **15**: 527–585.

Steggerda, F. R. and A. L. Soderwall, 1939. Relationship of the pars tuberalis to melanophore response in amphibia (*Rana pipiens*). *Jour. Cell. Comp. Physiol.*, **13**: 31–37.

Thörner, W., 1929. Beobachtungen über peripheren Kreislauf und Melanophoren unter dem Einfluss von Degeneration und Kationenwirkung in Durchspülungsversuchen am Frosch. *Pflüger's Arch. Ges. Physiol.*, **222**: 52–70.

Wyman, L. C., 1924. Blood and nerve as controlling agents in the movements of melanophores. *Jour. Exp. Zool.*, **39**: 73–132.

INDEX

ACETYLCHOLINE effects on permeability of erythrocytes, 311.
Acetylcholinesterase activity and ciliary action 347.
Adaptation to cold in arctic and tropical mammals and birds, 259.
Aeolosoma hemprichi (Ehr), cyst-formation in, 173.
Algae (blue-green), photosynthesis and photoreduction in, 157.
Algae, chlorophyll preparations from, 364.
Algae, nitrogen deficiency and coloration in, 360.
Algae, reproduction in Dudresnaya crassa Howe, 272.
Algae, respiration and iodine uptake, 370.
Alkaline phosphatase activity in kidneys of marine teleosts, 152.
Alkaline phosphatase activity in normal and scorbutic bones, 321.
Allen, Robert Day. Effect of Janus Green B on cleavage and protoplasmic viscosity, 353.
Amberson, William R., R. Dale Smith, Sylvia Himmelfarb, Carolyn Stout and Hans Hoch. Complex formation in protein solutions obtained by mild extraction of skeletal muscle, 314.
Amino acid effects on spermatozoa, 324.
Ammonium ion effects on marine microorganisms, 328.
Amphibian regeneration, 351.
Anderson, John Maxwell. A cytological and cytochemical study of the male accessory reproductive glands in the Japanese beetle, Popillia japonica Newman, 49.
Anderson, Thomas F. A new technique for the study of biological structures in the electron microscope, 315.
Androgenetic frog hybrids, 88.
Anfinsen, C. B. *See* Claude A. Villee, 322.
Anguilla rostrata, adaptive changes in chloride cells of, 439.
Annelid muscle, transphosphorylation in, 352.
Annual Report, 1.
Arbacia eggs, action of heparin on fertilization of, 340.
Arbacia eggs, effects of cortisone and desoxycorticosterone, 357.
Arbacia eggs, effect of P^{23} on division time of, 358.
Arbacia eggs, exchange of radioactive calcium, 339.
Arbacia eggs, phosphorylation of an enzyme system derived from, 333.
Arbacia eggs, photography of with infrared light, 360.
Arbacia eggs, photorecovery in, 342.
Arbacia eggs, respiration of, 335.
Arbacia eggs, temperature alteration of carbamate narcosis of, 338.
Arbacia gametes, viability in relation to time, 354.
Arbacia, inhibitor effects on mitotic phases of, 362.
Arbacia, jelly coat and membrane formation, 339.
Arbacia sperm, photorecovery in, 342.
Arctic mammals and birds, 225, 237, 259.
Atwood, K. C. Homology patterns of induced lethal mutations in Neurospora crassa, 332.
Auxin effects in Bryopsis, 369.
Axon (squid), observations on structure of, 345.
Axons, electrically induced changes in, 345.
Axoplasm, low frequency characteristics of, 344.

BALL, Eric G. and Octavia Cooper. Direct spectroscopic observations on cytochrome oxidase and its reaction with carbon monoxide, 317.
Barnes, T. Cunliffe and R. Beutner. Experiments supporting the phase-boundary theory of bio-electricity, 314.
Battley, Edwin H. *See* Albert Frenkel, 157.
Bean (kidney), control of elongation in hypocotyl of, 329.
Bees, orientation of by polarized light, 326.
Benaglia, Angelo E. *See* Milton Levy, 323.
Berman, Leonora. *See* Rudolf T. Kempton, 346.
Beutner, R, *See* T. Cunliffe Barnes, 314.
Bio-electricity, phase-boundary theory of, 314.
Bliss, Alfred F. Action of alcohol dehydrogenase on vitamin A, 330.
Blood-sugar concentration in crustaceans, 454.
Blowfly, carbohydrate taste thresholds and nutritive values in, 446.
Blum, Harold F. and Margie M. Mathews. Photorecovery after ultraviolet radiation in amphibian larvae, 330.

BLUM, HAROLD F., CHARLOTTE T. POPE, JUDITH P. PRICE and RALPH WITMER. Photochemical inactivation of chymotrypsin, 317.
BLUM, HAROLD F., J. COURTLAND ROBINSON and GORDON M. LOOS. Loci of action of ultraviolet and x-radiation, and of photorecovery, in the egg and sperm of Arbacia, 342.
Body insulation in mammals and birds, 225.
BOETTIGER, EDWARD G. and EDWIN FURSHPAN. Observations on the flight motor of Diptera, 346.
Bones, phosphatase activity in, 321.
BONNER, JOHN TYLER. Observations on polarity in the slime mold Dictyostelium discoideum, 143.
BOWDEN, BERNARD J. Some observations on a luminescent freshwater limpet from New Zealand, 373.
BRIDGMAN, ANNA JOSEPHINE. Notes on growth and cystment in Dileptus binucleatus, 348.
BROWNE, MARIE J., MARJORIE W. PITTS and ROBERT F. PITTS. Alkaline phosphatase activity in kidneys of glomerular and aglomerular marine teleosts, 152.
Bryopsis, auxin effects in, 369.
BUCK, JOHN B. and MARGARET L. KEISTER. Experiments on spiracles, 315.
BULLOCK, THEODORE HOLMES, MELVIN COHEN and DYREL FAULSTICK. Effect of stretch on conduction in single nerve fibers, 320.
BURBANCK, W. D. Growth of pedigreed strains of Paramecium caudatum and P. aurelia on a non-living medium, 353.
BURNS, JEAN and D. EUGENE COPELAND. Chloride excretion in head region of Fundulus heteroclitus, 381.

CALCIUM (radioactive), exchange of by Arbacia eggs, 339.
Campanularia colonies, growth of, 357.
Carbohydrate influence on potassium content of leucocytes and muscle, 312.
Carbohydrate metabolism of invertebrate tissues, 365.
Carbohydrate metabolism in mammalian muscle, 312.
Carbohydrate taste thresholds and nutritive values in the Blowfly, 446.
Carbon monoxide, cytochrome oxidase reaction with, 317.
Cell division as affected by tissue extracts, 340.
Cell division, changes in protoplasmic cortex during, 341.
Cell division, x-ray effects on mechanisms of, 317.
Cercaria parvicaudata Stunkard and Shaw (1931), observations on, 136.
Chaetopterus egg, breakdown of the germinal vesicle, 363.
Chaetopterus egg, sensitivity of to ultraviolet light, 359.
Chaetopterus egg, study of maturation in, 341.
CHAMBERS, EDWARD L. and THOMAS MENDE. The role of phosphagen in fertilization of the sea urchin egg, 316.
CHAMBERS, ROBERT and CHIEN-YUAN KAO. Micro-injection of calcium chloride into the giant nerve axon of the squid, 344.
CHANCE, BRITTON. Oxidase activity—light-absorption relationships in cytochrome system of heart muscle preparations, 318.
CHASE, AURIN M. On the nature of Cypridina luciferin, 326.
CHENEY, RALPH HOLT. Viability of Arbacia gametes in relation to time, 354.
Chick embryo, nutritional requirements of, 120.
Chloride cell changes in Anguilla rostrata, 439.
Chloride excretion in head region of Fundulus, 381.
Chloroplasts, oxidation and reductions catalyzed by, 318.
Chloroplast reactions, 327.
Chlorophyll preparations from blue-green algae, 364.
Chromatophores of vertebrates, pituitary effects on, 487.
Chromosomal variations in larvae of Triturus torosus, 386.
Chymotrypsin, photochemical inactivation of, 317.
Ciliary action dependence on acetylcholinesterase activity, 347.
Ciliates, marine sand-dwelling, 349.
CLARK, A. M. *See* D. S. GROSCH, 359.
CLOWES, G. H. A., A. K. KELTCH, PHILIPP STRITTMATTER and C. P. WALTERS. Action of dinitrocarvacrol and dinitrothymol on respiration of fertilized Arbacia and Mactra eggs and certain mammalian tissues and cell-free, particulate, phosphorylating systems, 335.
CLOWES, G. H. A. *See* A. K. KELTCH, 334.
CLOWES, G. H. A. *See* PHILIPP STRITTMATTER, 333.
Coelomic fluid of Thyone Briareus (Lesueur), 343.
COHEN, MELVIN. *See* THEODORE HOLMES BULLOCK, 320.
COHEN, SEYMOUR S. Studies on the distribution of the oxidative pathway of glucose-6-phosphate utilization, 369.
Commensal hydroid from California, 74.

Conalbumin preparation and properties, 323.
Conduction in single nerve fibers, 320.
COOPER, OCTAVIA. *See* ERIC G. BALL, 317.
COOPERSTEIN, S. J. and ARNOLD LAZAROW. Comparative studies on the cytochrome oxidase and succinic dehydrogenase content of toadfish (Opsanus tau) and rat kidneys, 356.
COOPERSTEIN, S. J. and ARNOLD LAZAROW. Cytochrome oxidase and succinic dehydrogenase contents of squid (Loligo pealii) axoplasm and other nervous tissue, 356.
COOPERSTEIN, S. J. and ARNOLD LAZAROW. Reduction of Janus Green by isolated enzyme systems, 321.
COOPERSTEIN, S. J. *See* ARNOLD LAZAROW, 322, 361.
COPELAND, D. EUGENE. *See* JEAN BURNS, 381.
CORNMAN, IVOR. Dividing Arbacia eggs as test objects for the effects of cortisone and desoxycorticosterone on cell division, potassium permeability, and glucose utilization, 357.
CORNMAN, IVOR. Selective damage to fibroblasts by desoxycorticosterone in cultures of mixed tissues, 328.
CORNMAN, IVOR. Temperature alteration of carbamate narcosis of dividing Arbacia eggs, 338.
Cortisone effects on Arbacia eggs, 357.
COSTELLO, D. P. and CATHERINE HENLEY. Heteroploidy in Triturus torosus. II. The incidence of chromosomal variations in shipped larvae, 386.
CROWELL, SEARS and MALCOLM RUSK. Growth of Campanularia colonies, 357.
Crustaceans, blood-sugar studies in, 454.
CURTIS, HOWARD J. and JOHN L. MYERS. Failure to find intercellular protoplasmic continuity in Griffithsia globulifera, 350.
Cypridina luciferin, 326.
Cyst-formation in Aeolosoma hemprichi (Ehr), 173.
Cytochemical study of male accessory reproductive glands in the Japanese beetle, Popillia japonica Newman, 49.
Cytochrome oxidase contents of squid axoplasm and other nervous tissues, 356.
Cytochrome oxidase contents of toadfish and rat kidneys, 356.
Cytochrome oxidase of islet tissue of the toadfish, 361.
Cytochrome oxidase reaction with carbon monoxide, 317.
Cytochrome system of heart muscle preparations, 318.
Cytological study of male accessory reproductive glands in the Japanese beetle, Popillia japonica Newman, 49.
Cytoplasmic component action on jelly coat and membrane formation in Arbacia, 339.

DAN, JEAN CLARK. Fertilization in the Medusan, Spirocodon saltatrix, 412.
DAN, JEAN CLARK. Sperm entrance in Echinoderms, observed with the phase contrast microscope, 399.
DANIELLI, J. F. Studies on spindle material, 369.
DEKORNFELD, T. J. *See* A. M. SHANES, 327.
Denaturation of several proteins, 336, 337.
Desoxycorticosterone damage to fibroblasts, 328.
Desoxycorticosterone effects on Arbacia eggs, 357.
Desoxypentose nucleic acid, 336.
Detergent activation of Nereis eggs. 362.
DETHIER, V. G. *See* C. C. HASSETT, 446.
Diadema antillarum Philippi, sensitivity of, 329.
Dictyostelium discoideum, observations on polarity in, 143.
Differentiation in the early chick embryo, 120.
Dileptus binucleatus, growth and cystment, 348.
Dinitrocarvacrol action on Arbacia and Mactra eggs, 335.
Dinitrothymol action on Arbacia and Mactra eggs, 335.
Diptera, flight motor of, 346.
Drosophila, flight sounds of, 300.
Dudresnaya crassa Howe, 272.

EARTHWORM regeneration, 425.
Echinarachnius parma eggs, action of heparin on fertilization of, 340.
Echinoderm phosphatases, 370.
Echinoderms, sperm entrance in, 399.
Ecological significance of glycogen depletion in a nemertean, 469.
Elasmobranch, erythrocytes of, 351.
Elasmobranch, musculature development of, 364.
Electrolyte pump in isolated surviving frog skin, 313.
Electron microscope studies of biological structures, 315.
ELLIOTT, ALFRED M. and ROBERT L. HUNTER. Phosphatase activity in Tetrahymena, 357.
ELLIOTT, ALFRED M. *See* JAY S. ROTH, 335.
Elongation in the hypocotyl of the kidney bean, 329.
Embryo (chick), nutritional requirements of, 120.
Enzyme contents of islet tissue of toadfish, 361.
Enzyme contents of squid, 356.
Enzyme contents of toadfish and rat kidneys, 356.

Enzymes in eggs of Mactra, 367.
Enzyme reduction of Janus Green, 321.
Enzyme system from Arbacia eggs, phosphorylation of, 333.
Enzymatic conversion of ovalbumin to plakalbumin, 322.
Erythrocytes of dogfish, mackerel and tautog, 338.
Erythrocytes of Elasmobranchs, 351.
Erythocyte, ionic exchanges and equilibria, 311.
Erythrocytes, permeability at different temperatures, 325.
Erythrocyte permeability to physostigmine and acetylcholine, 311.
Erythrocytes, potassium accumulation in, 310.
Erythrocytes, potassium permeability, 311.
Escherichia coli, population dynamics in, 331.
Estrone, action of on nuclear volume, 112.
Euphausiacea relationship to the scattering layer, 181.
EVANS, TITUS C. Some effects of x-rays on the mechanism of cell division, 317.

FAULSTICK, DYREL. *See* THEODORE HOLMES BULLOCK, 320.
Faunistic survey of the Nudibranchiata of New England, 352.
FAURÉ-FREMIET, E. The marine sand-dwelling ciliates of Cape Cod Shores, 349.
Fertilization in Spirocodon saltatrix, 412.
Fibroblasts, selective damage to, 328.
FIELD, JOHN. *See* C. N. PEISS, 213.
Fish, respiratory metabolism in, 213.
Fish, temperature acclimatization in, 416.
FISHER, K. C. *See* J. F. MANERY, 312.
Flight motor of Diptera, 346.
Flight sounds of Drosophila, 300.
FREEMAN, JOHN A. Oxygen consumption, brain metabolism and respiratory movements of goldfish during temperature acclimatization, with special reference to lowered temperatures, 416.
FRENKEL, ALBERT, HANS GAFFRON and EDWIN H. BATTLEY. Photosynthesis and photoreduction by the blue-green alga, Synechococcus elongatus, Näg, 157.
Frog hybrids, development of, 88.
Frog skin, electrolyte pump in, 313.
Fundulus, chloride excretion in head region of, 381.
Fundulus egg, some physical constants of, 337.
Fundulus, neurosecretory material from, 371.
FURSHPAN, EDWIN. *See* EDWARD G. BOETTIGER, 346.

GAFFRON, HANS. *See* ALBERT FRENKEL, 157.
GAFFRON, HANS. *See* ALAN H. MEHLER, 318.
GAGNON, ANDRÉ. Action of heparin on fertilization and cleavage in some marine eggs, 341.
GALAMBOS, ROBERT. *See* CARROLL M. WILLIAMS, 300.
GANS, J. *See* C.C. HASSETT, 446.
GARREY, W. E. "Spot" beats in deganglionated Limulus hearts, 358.
GATES, G. E. Regeneration in an earthworm, Eisenia foetida (Savigny) 1826. III. Regeneration from simultaneous anterior and posterior transections, 425.
Germinal vesicle breakdown in Chaetopterus eggs, 363.
GETMAN, HERBERT C. Adaptive changes in the chloride cells of Anguilla rostrata, 439.
GIESE, ARTHUR C. *See* PATRICK H. WELLS, 163.
GILMAN, LAUREN C. Position of Japanese varieties of Paramecium caudatum with respect to American varieties, 348.
GLASER, OTTO. Some physical constants of the Fundulus egg, 337.
GLASSMAN, H. N. *See* M. H. JACOBS, 325.
Glycogen depletion during starvation in Micrura leidyi (Verrill), 469.
Glycolytic factor influence on potassium and sodium content of the yeast cell, 319.
Goldfish, temperature acclimatization in, 416.
GOLDSTEIN, LESTER. An experimental study of maturation in Chaetopterus eggs, 341.
GOURLEY, D. R. H. *See* J. F. MANERY, 312.
GREEN, JAMES W. and JOSEPH F. HOFFMAN. Isotonic solutions for the erythrocytes of the smooth dogfish, mackerel and tautog, 338.
GREEN, JAMES W. and A. K. PARPART. Effect of metabolic poisons on potassium loss from rabbit red cells, 325.
GREEN, JAMES W. and JAY S. ROTH. The effect of P^{32} on the division time of Arbacia eggs, 358.
GREIG, MARGARET E. The effect of physostigmine and acetylcholine on the permeability of erythrocytes, 311.
GRIFFIN, DONALD R. Polarized light and the orientation of bees, 326.
Griffithsia globulifera, protoplasmic continuity in, 350.
GROSCH, D. S. Staining procedures for whole mounts of the spinning glands of Habrobracon larvae, 358.
GROSCH, D. S. Starvation studies with the parasitic wasp Habrobracon, 65.
GROSCH, D. S. and A. M. CLARK. Starvation studies with Habrobracon. II. Comparison in longevity, 359.

GROSS, PAUL RANDOLPH. Variable sensitivity of Chaetopterus eggs to ultraviolet light, 359.
GROSS, SAMSON R. Heterokaryosis between opposite mating types of Neurospora crassa, 331.
Growth and cystment in Dileptus binucleatus, 348.

HABROBRACON larvae, staining procedures for whole mounts of the spinning glands of, 358,
Habrobracon, starvation studies with, 65, 359.
HAND, CADET and JOHN R. HENDRICKSON. A two-tentacled, commensal hydroid from California, 74.
HARDING, CLIFFORD V. The action of heparin on fertilization in the eggs of Arbacia punctulata and Echinarachnius parma, 340.
HARDING, D. *See* L. V. HEILBRUNN, 340.
HARVEY, ETHEL BROWNE and GEORGE I. LAVIN. Photography of Arbacia punctulata eggs with infrared light, 360.
HARVEY, E. NEWTON. Luminescent reactions in the "railroad worm," Phrixothrix, 360.
HASSETT, C. C., V. G. DETHIER and J. GANS. A comparison of nutritive values and taste thresholds of carbohydrates for the Blowfly, 446.
HASTINGS, A. BAIRD. *See* CLAUDE A. VILLEE, 312.
HAVEL, V. J. *See* L. H. KLEINHOLZ, 454.
HAXO, FRANCIS and PHYLLIS STROUT. Nitrogen deficiency and coloration in red algae, 360.
HAXO, FRANCIS. *See* JULIUS SILBERGER, 364.
HAY, ELIZABETH DEXTER. Role of epidermis in amphibian regeneration as revealed by triploid and haploid transplants to diploid limbs, 351.
HAYWARD, HUGH R. *See* GEORGE T. SCOTT, 363.
Heat regulation in some arctic and tropical mammals and birds, 237.
HEILBRUNN, L. V., W. L. WILSON and D. HARDING. Effect of tissue extracts on cell division, 340.
HENDRICKSON, JOHN R. *See* CADET HAND, 74.
HENLEY, CATHERINE. *See* D. P. COSTELLO, 386.
Heparin action on some marine eggs, 340, 341.
HERLANT-MEEWIS, HENRIETTE. Cyst-formation in Aeolosoma hemprichi (Ehr), 173.
Heterokaryosis in Neurospora crassa, 331.
Heteroploidy in Triturus torosus, 386.
HIMMELFARB, SYLVIA. *See* WILLIAM R. AMBERSON, 314.
HOCH, HANS. *See* WILLIAM R. AMBERSON, 314.
HOCK, RAYMOND. *See* P. F. SCHOLANDER, 225, 237, 259.
HOFFMAN, JOSEPH F. *See* JAMES W. GREEN, 338.
Homology patterns in Neurospora crassa, 332.
Hormone action on nuclear volume, 112.
HUF, ERNST G. The nature of the electrolyte pump in the isolated surviving frog skin, 313.
HUNTER, ROBERT L. *See* ALFRED M. ELLIOTT, 357.
HUNTER, ROBERT L. *See* JAY S. ROTH, 335.
HUTNER, SEYMOUR H. *See* WOLF VISHNIAC, 328.
Hydra, reproductive potential in Pelmatohydra oligactis, 285.
Hydranth formation in Tubularia, 361.
Hydroid from California, 74.
Hyperglycemia and regulatory mechanisms in crustaceans, 454.

INHIBITOR effects on the early chick embryo, 120.
Inhibitor effects on mitotic phases in Arbacia, 362.
Inhibitor influence on egg cells of Mactra, 363.
Insulation in mammals and birds, 225, 237, 259.
Insulin effects on potassium content of leucocytes and muscle, 312.
Invertebrate photoreceptors, 342.
Ion effects on Echinoderm phosphatases, 370.
Ion effects of electrical changes in single axons, 345.
Ionic balance required by some marine microorganisms, 328.
Ionic exchanges and equilibria of the erythrocyte, 311.
IRVING, LAURENCE. *See* P. F. SCHOLANDER, 225, 237, 259.
Isotonic solutions for erythrocytes of dogfish, mackerel and tautog, 338.

JACOBS, M. H. Some ionic exchanges and equilibria of the erythrocyte, 311.
JACOBS, M. H. and H. N. GLASSMAN. Permeability of erythrocytes at different temperatures, 325.
JACOBS, WILLIAM P. Control of elongation in the hypocotyl of the kidney bean, 329.
JACOBS, WILLIAM P. Auxin effects in Bryopsis, 369.
JACOBSON, MARC. *See* GEORGE T. SCOTT, 319.
Janus Green B effects on cleavage and protoplasmic viscosity, 353.
Janus Green reduction by isolated enzyme systems, 321.

Janus Green reduction by liver cell constituents, 322.
Japanese beetle, reproductive glands in, 49.
JOHNSON, FRED. *See* P. F. SCHOLANDER, 237.

KAHN, ARTHUR J. and ALEXANDER SANDOW. Effects of nitrate on muscle and nerve responses, 316.
KAO, CHIEN-YUAN. *See* ROBERT CHAMBERS, 344.
Karyometric-statistical method as used for nuclear volume studies, 112.
KEISTER, MARGARET L. *See* JOHN B. BUCK, 315.
KELLY, SALLY. Respiration and iodine uptake by the brown algae, 370.
KELTCH, A. K., PHILIPP STRITTMATTER, C. P. WALTERS and G. H. A. CLOWES. Action of a series of nitro- and halophenols on the respiration and cell division of Mactra solidissima, 334.
KELTCH, A. K. *See* G. H. A. CLOWES, 335.
KELTCH, A. K. *See* PHILLIPP STRITTMATTER, 333.
KEMPTON, RUDOLF T. and LEONORA BERMAN. Renal chloride excretion by the smooth dogfish, Mustelus canis, 346.
Kidneys of marine teleosts, phosphatase activity in, 152.
KIND, C. ALBERT. Effect of divalent ions on Echinoderm phosphatases, 370.
KISLIUK, ROY. *See* WOLF VISHNIAC, 328.
KLEINHOLZ, L. H., V. J. HAVEL and R. REICHART. Studies in the regulation of blood-sugar concentration in crustaceans. II. Experimental hyperglycemia and the regulatory mechanisms, 454.

LALOR FELLOWSHIP REPORTS, 369.
Larvae (amphibian), photorecovery of, 330.
LAVIN, GEORGE I. Microscopy in the ultraviolet visible and infrared, 342.
LAVIN, GEORGE I. *See* ETHEL BROWNE HARVEY, 360.
LAZAROW, ARNOLD and S. J. COOPERSTEIN. Enzymes of the isolated islet tissue of the toadfish (Opsanus tau): cytochrome oxidase and succinic dehydrogenase, 361.
LAZAROW, ARNOLD and S. J. COOPERSTEIN. Reduction of Janus Green by liver cell constituents and a proposed mechanism for the supravital staining of mitochondria, 322.
LAZAROW, ARNOLD. *See* S. J. COOPERSTEIN, 321, 356.
LEBARON, GEORGE, JR. *See* ALLAN SCOTT, 363.
Leucocytes, potassium content of, 312.
LEVY, MILTON and ANGELO E. BENAGLIA. The denaturation of ricin as affected by pH and temperature, 323.
LEVY, MILTON and ROBERT C. WARNER. Effect of pH upon the rate of denaturation of several proteins, 337.
LEVY, MILTON. *See* ROBERT C. WARNER, 336.
LICHTENSTEIN, ROBERT. *See* CLAUDE A. VILLEE, 365, 366.
Limpet, observations on luminescence, 373.
Limulus hearts, "spot" beats in, 358.
LINDERSTRØM-LANG, K. *See* CLAUDE A. VILLEE, 322.
Liver cell reduction of Janus Green, 322.
LOOS, GORDON M. *See* HAROLD F. BLUM, 342.
Luciferin (Cypridina), 326.
Luminescent freshwater limpet, observations on, 373.
Luminescent reaction in Phrixothrix, 360.

MACTRA eggs, inhibitor influence on, 363.
Mactra eggs, enzymes in, 367.
Mactra eggs, respiration and cell division of, 334, 335.
MANERY, J. F., K. C. FISHER, D. R. H. GOURLEY, D. L. WILSON and M. F. J. TAYLOR. The influence of carbohydrates and insulin on the potassium content of leucocytes and muscle, 312.
Marine eggs, action of heparin on, 341.
Marine microorganisms, ionic balance of, 328.
MARSHAK, ALFRED. Comparison of the base composition of nucleic acids of nuclei and cytoplasm of different mammalian tissues, 332.
MATHEWS, MARGIE M. *See* HAROLD F. BLUM, 330.
Mechanism of accumulation, 308.
MEHLER, ALAN H. Reactions catalyzed by isolated chloroplasts, 327.
MEHLER, ALAN H. Reactions related to photosynthesis and respiration, 371.
MEHLER, ALAN H. and HANS GAFFRON. Oxidation and reductions catalyzed by isolated chloroplasts, 318.
MENDE, THOMAS. *See* EDWARD L. CHAMBERS, 316.
Metabolic poison effects on potassium loss from rabbit red cells, 325.
Metacercariae encysted in Limulus polyphemus, 347.
Micro-injection of calcium chloride into squid axon, 344.
Microscopy in the ultraviolet visible and infrared, 342.
Micrura leidyi (Verrill), glycogen depletion during starvation, 469.
MILLAR, F. K. *See* F. C. STEWARD, 309.

Miller, James A. Are nutritive substances essential for hydranth formation in Tubularia?, 361.

Millott, Norman. The sensitivity to light, reactions to shading, pigmentation, and color change of the sea urchin, Diadema antillarum Philippi, 329.

Millott, Norman. Integumentary pigmentation and the coelomic fluid of Thyone Briareus (Lesueur), 343.

Milne, Lorus J. and Margery J. Milne. Invertebrate photoreceptors, 342.

Mitochondria, proposed mechanism for supravital staining of, 322.

Monroy, Alberto and John Runnström. Further studies on the action of a cytoplasmic component on jelly coat and membrane formation in Arbacia punctulata, 339.

Moore, Anna-Betty Clark. The development of reciprocal androgenetic frog hybrids, 88.

Moore, George M. Progress report on investigations of the Nudibranchiata of New England, 352,

Moore, Hilary B. The relation between the scattering layer and the Euphausiacea, 181.

Morphogenesis in the early chick embryo, 120.

Morphology, water and biological, 367.

Muscle, carbohydrate metabolism in, 312.

Muscle (heart), cytochrome system of, 318.

Muscle, potassium content of, 312.

Muscle (skeletal), protein solutions from, 314.

Muscle response to nitrate, 316.

Musculature development in Elasmobranchs, 364.

Mustelus canis, notes on early development of, 474.

Mustelus canis, renal chloride excretion by, 346.

Myers, John L. *See* Howard J. Curtis, 350.

Mytilus, penetration of phosphate into gills of, 319.

NATHANSON, Neal. *See* Claude A. Villee, 365, 366.

Nereis egg, activation of by a detergent, 362.

Nereis egg, solubility of components, 362.

Nereis limbata, vascular patterns and behavior, 350.

Nereis virens, vascular patterns and behavior, 350.

Nerve, drug and ion action in, 309.

Nerve fibers, conduction in, 320.

Nerve, low frequency characteristics of, 344.

Nerve, progressive changes in admittance after dissection, 344.

Nerve response to nitrate, 316.

Nerves, spectrophotometric observations on, 327.

Neurosecretory material from Fundulus, 371.

Neurospora crassa, heterokaryosis between opposite mating types of, 331.

Neurospora crassa, homology patterns in lethal mutations, 332.

Nicoll, Paul A. Vascular patterns and behavior in typical parapodia of Nereis virens and Nereis limbata, 350.

Nitrate effects on muscle and nerve responses, 316.

Nitrogen deficiency and coloration in red algae, 360.

Nuclear volume, action of estrone and progesterone on, 112.

Nucleic acids, comparison of base composition of, 112.

Nudibranchiata of New England, 352.

Nutritional requirements of the early chick embryo, 120.

OSCILLOSCOPIC analysis of flight sounds of Drosophila, 300.

Osterhout, W. J. V. Activation of Nereis eggs by a detergent, 362.

Osterhout, W. J. V. The mechanism of accumulation, 308.

Osterhout, W. J. V. Relative solubility of the components of the Nereis egg, 362.

Ottesen, M. *See* Claude A. Villee, 322.

Ovalbumin conversion to plakalbumin, 322.

Ovulation in Mustelus canis, 474.

Oxidation and reductions catalyzed by isolated chloroplasts, 318.

Oxidative pathway of glucose-6-phosphate utilization, 369.

PARAMECIUM calkinsi, occurrence of a new variety, 366.

Paramecium caudatum, Japanese and American varieties, 348.

Paramecium growth on a non-living medium, 353.

Paramecium, method for obtaining large numbers at fission-stage, 366.

Parpart, A. K. Absence of a fine internal network in erythrocytes of Elasmobranchs, 351.

Parpart, A. K. *See* James W. Green, 325.

Peiss, C. N. and John Field. Respiratory metabolism of excised tissues of warm and cold-adapted fishes, 213.

Pelmatohydra oligactis, reproductive potential of a single clone, 285.

Peptide effects on spermatozoa, 324.

Permeability of erythrocytes at different temperatures, 325.

Permeability of erythrocytes to physostigmine and acetylcholine, 311.
Permeability of human erythrocytes to potassium, 311.
pH effects on denaturation of proteins, 337.
pH effects on denaturation of ricin, 323.
Phase-boundary theory of bio-electricity, 314.
Phosphagen in fertilization of the sea urchin egg, 316.
Phosphatase activity in bones, 321.
Phosphatase activity in marine teleosts, 152.
Phosphatase activity in Tetrahymena, 335, 357.
Phosphate penetration into gills of Mytilus, 319.
Phosphorus (P^{32}) effects on division time of Arbacia eggs, 358.
Phosphorus metabolism in invertebrate tissues, 366.
Phosphorylating systems, action of dinitrocarvacrol and dinitrothymol on, 335.
Phosphorylation of an enzyme system derived from Arbacia eggs, 333.
Photochemical inactivation of Chymotrypsin, 317.
Photography of Arbacia eggs with infrared light, 360.
Photoreactivation in Strongylocentrotus purpuratus, 163.
Photoreceptors (invertebrate), 342.
Photorecovery in amphibian larvae, 330.
Photorecovery in Arbacia eggs and sperm, 342.
Photoreduction in a blue-green alga, 157.
Photosynthesis in a blue-green alga, 157.
Photosynthetic reactions, 371.
Phrixothrix, luminescent reactions in, 360.
Physical constants of the Fundulus egg, 337.
Physostigmine effects on permeability of erythrocytes, 311.
Pigmentation in Thyone Briareus (Lesueur), 343.
Pitts, Marjorie W. *See* Marie J. Browne, 152.
Pitts, Robert F. *See* Marie J. Browne, 152.
Pituitary effects on vertebrate chromatophores, 487.
Plant cells, growth and metabolism in, 309.
Polarity in the slime mold Dictyostelium discoideum, 143.
Polarized light and the orientation of bees, 326.
Pope, Charlotte T. *See* Harold F. Blum, 317.
Popillia japonica Newman, reproductive glands in, 49.
Population dynamics in Escherichia coli, 331.
Potassium accumulation in human erythrocytes, 310.
Potassium content of leucocytes and muscle, 312.
Potassium content of the yeast cell, 319.
Potassium loss from rabbit red cells, 325.
Potassium movement in relation to drug and ion action in nerve, 309.
Potassium permeability in human erythrocytes, 311.
Price, Judith P. *See* Harold F. Blum, 317.
Progesterone, action of on nuclear volume, 112.
Proteins, kinetics of denaturation of, 336.
Proteins, pH effects on denaturation of, 337.
Protein solutions from skeletal muscle, 314.
Protoplasmic continuity in Griffithsia globulifera, 350.
Protoplasmic cortex changes during cell division, 341.

RABBIT red cells, potassium loss from, 325.
Regeneration in amphibians, 351.
Regeneration in an earthworm, 425.
Reichart, R. *See* L. H. Kleinholz, 454.
Reid, W. Malcolm. Glycogen depletion during starvation in the nemertean Micrura leidyi (Verrill) and its ecological significance, 469.
Renal alkaline phosphatase, 152.
Renal chloride excretion by Mustelus canis, 346.
Reproduction of Dudresnaya crassa Howe, 272.
Reproductive glands in the Japanese beetle, Popillia japonica Newman, 49.
Reproductive potential of a single clone of Pelmatohydra oligactis, 285.
Respiration (brown algae), 370.
Respiration (Mactra solidissima), 334.
Respiration (sea urchin sperm), 324.
Respiratory metabolism in fish, 213.
Rich, Alexander. Double refraction of flow studies on desoxypentose nucleic acid, 336.
Ricin denaturation, 323.
Robinson, J. Courtland. *See* Harold F. Blum, 342.
Rolander, Brita. *See* Claude A. Villee, 365, 366.
Ronkin, R. R. Anomalous penetration of phosphate into gills of Mytilus, 319.
Roth, Jay S., Alfred M. Elliott and Robert L. Hunter. Some observations on phosphate uptake by Tetrahymena geleii, 335.
Roth, Jay S. *See* James W. Green, 358.
Rudenberg, F. Hermann. Exchange of radioactive calcium by unfertilized and fertilized Arbacia punctulata eggs with or without jelly coats, 339.
Runnström, John. *See* Alberto Monroy, 339,
Runnström, John. *See* Erik Vesseur, 324.
Rusk, Malcolm. *See* Sears Crowell, 357.

SALVATORE, CARLOS ALBERTO. Action of estrone and progesterone on nuclear volume (studied by applying the Karyometric-statistical method), 112.

SANDOW, ALEXANDER. *See* ARTHUR J. KAHN, 316.

Scattering layer relationships to Euphausiacea, 181.

SCHMITT, OTTO H. Some low frequency characteristics of axoplasm and the nerve membrane, 344.

SCHNEIDER, LILLIAN K. Population dynamics in Escherichia coli, 331.

SCHOLANDER, P. F., VLADIMIR WALTERS, RAYMOND HOCK and LAURENCE IRVING. Body insulation of some artic and tropical mammals and birds, 225.

SCHOLANDER, P. F., RAYMOND HOCK, VLADIMIR WALTERS, FRED JOHNSON and LAURENCE IRVING. Heat regulation in some arctic and tropical mammals and birds, 237.

SCHOLANDER, P. F., RAYMOND HOCK, VLADIMIR WALTERS and LAURENCE IRVING. Adaptation to cold in arctic and tropical mammals and birds in relation to body temperature, insulation, and basal metabolic rate, 259.

SCOTT, ALLAN. Cytological analysis of the effects of cyanide and 4,6-dinitro-orthocresol on the mitotic phases in Arbacia punctulata, 362.

SCOTT, ALLAN and GEORGE LEBARON, JR. Factors involved in the breakdown of the germinal vesicle in the egg of Chaetopterus pergamentaceus, 363.

SCOTT, GEORGE T. and HUGH R. HAYWARD. Influence of the glycolytic inhibitor iodoacetic acid on aging and on the potassium and sodium content of the egg cells of Mactra solidissima, 363.

SCOTT, GEORGE T. and MARC JACOBSON. The influence of glycolytic factors on the potassium and sodium content of the yeast cell, 319.

Sea urchin (Diadema antillarum Philippi), sensitivity of, 329.

Sea urchin egg, phosphagen in fertilization of, 316.

Sea urchin, photoreactivation in gametes of, 163.

Sea urchin sperm, respiration and fertilizing capacity of, 324.

SEAMAN, GERALD R. Dependence of ciliary action upon acetylcholinesterase activity, 347.

SEMINAR PAPERS PRESENTED DURING THE SUMMER OF 1950, 321.

SHANES, A. M. Potassium movement in relation to drug and ion action in nerve, 309.

SHANES, A. M. and T. J. DEKORNFELD. Some spectrophotometric observations on invertebrate nerves and their extracts, 327.

SHEPPARD, C. W. The disturbance by x-rays of selective potassium accumulation in human erythrocytes, 310.

SILBERGER, JULIUS and FRANCIS HAXO. Aqueous chlorophyll preparations from blue-green algae, 364.

SMITH, R. DALE. *See* WILLIAM R. AMBERSON, 314.

SMITH, STUART W. Separation and characterization of neurosecretory material from Fundulus heteroclitus, 371.

SOCIETY OF GENERAL PHYSIOLOGISTS, PAPERS PRESENTED AT MEETING OF, 308.

Sodium content of the yeast cell, 319.

SOLOMON, SIDNEY and JULIAN M. TOBIAS. Preliminary observations on squid axon structure. Light scattering properties using an intracellular light source and mechanical prod, 345.

Solubility of the components of the Nereis egg, 362.

Spectrophotometric observations on nerves, 327.

Spectroscopic observations on cytochrome oxidase and its reaction with carbon monoxide, 317.

Sperm entrance in Echinoderms, 399.

Sperm (sea urchin), respiration and fertilizing capacity of, 324.

Spermatozoa, extension of functional life span, 324.

Spindle material studies, 369.

Spiracles, experiments on, 315.

Spirocodon saltatrix, fertilization in, 412.

SPRATT, NELSON T., JR. Nutritional requirements of the early chick embryo. III. The metabolic basis of morphogenesis and differentiation as revealed by the use of inhibitors, 120.

Squid axon, micro-injection of calcium chloride into, 344.

Squid axon structure, 345.

Squid axoplasm, enzyme distribution in, 356.

Staining procedures for whole mounts of the spinning glands of Habrobracon larvae, 358.

Starvation studies with Habrobracon, 65, 359.

STEINBERG, D. *See* CLAUDE A. VILLEE, 322.

STEWARD, F. C. and F. K. MILLAR. The role of growth and metabolism in ion accumulation of plant cells, 309.

STEWART, PETER A. Progressive changes in the nerve membrane admittance after dissection, 344.
STOUT, CAROLYN. *See* WILLIAM R. AMBERSON, 314.
STRAUS, WILLIAM L., JR. A study of the development of the fin and trunk musculature in Elasmobranch fishes, 364.
Stretch effects on conduction in single nerve fibers, 320.
STRITTMATTER, PHILIPP, A. K. KELTCH, C. P. WALTERS and G. H. A. CLOWES. Oxidative phosphorylation by a cell-free, particulate, enzyme system derived from fertilized Arbacia eggs, 333.
STRITTMATTER, Philipp. *See* G. H. A. CLOWES, 335.
STRITTMATTER, PHILIPP. *See* A. K. KELTCH, 334.
Stroboscopic analysis of flight sounds of Drosophila, 300.
Strongylocentrotus purpuratus, photoreactivation in, 163.
STROUT, PHYLLIS. *See* FRANCIS HAXO, 360.
STUNKARD, HORACE W. Further observations on Cercaria parvicaudata Stunkard and Shaw (1931), 136.
STUNKARD, HORACE W. Larval trematodes from the plananian, Dugesia tigrinum, 347.
STUNKARD, HORACE W. Microphallid metacercariae encysted in Limulus polyphemus, 347.
Succinic dehydrogenase contents of squid axoplasm and other nervous tissues, 356.
Succinic dehydrogenase content of toadfish and rat kidneys, 356.
Succinic dehydrogenase content of islet tissues of the toadfish, 361.

TAYLOR, I. M. and J. M. WELLER. Studies on the permeability of human erythrocytes to potassium, 311.
TAYLOR, M. F. J. *See* J. F. MANERY, 312.
TAYLOR, WM. RANDOLPH. Reproduction of Dudresnaya crassa Howe, 272.
Teleosts (marine), phosphatase activity in kidneys of, 152.
Temperature acclimatization in goldfish, 416.
Temperature alteration of carbamate narcosis of dividing Arbacia eggs, 338.
Temperature effects on denaturation of ricin, 323.
Temperature effects on erythrocyte permeability, 325.
Tetrahymena geleii, phosphate uptake by, 335.
Tetrahymena, phosphatase activity in, 357.
TEWINKEL, LOIS E. Notes on ovulation, ova, and early development in the smooth dogfish, Mustelus canis, 474.
Thyone Briareus (Lesueur), pigmentation and coelomic fluid of, 343.
Tissue extract effects on cell division, 340.
Tissues (invertebrate), carbohydrate metabolism in, 365.
Tissues (invertebrate), phosphorus metabolism in, 366.
Tissues (mammalian), composition of nucleic acids of, 332.
TOBIAS, JULIAN M. Electrically induced optical and dimensional changes in single axons including the squid. Preliminary observations on ion effects, 345.
TOBIAS, JULIAN M. *See* SIDNEY SOLOMON, 345.
Transphosphorylation in annelid muscle, 352.
Trematodes from the planarian, Dugesia tigrinum, 347.
Triturus torosus, heteroploidy in, 386.
Tropical mammals and birds, 225, 237, 259.
Tubularia, hydranth formation in, 361.
TURNER, C. L. The reproductive potential of a single clone of Pelmatohydra oligactis, 285.
TYLER, ALBERT. Extension of the functional life span of spermatozoa by amino acids and peptides, 324.

ULTRAVIOLET light injury in Strongylocentrotus purpuratus, 163.
Ultraviolet light, sensitivity of Chaetopterus eggs to, 359.
Ultraviolet microscopy, 342.
Ultraviolet radiation effects on amphibian larvae, 330.

VASSEUR, ERIK, ELSA WICKLUND and JOHN RUNNSTRÖM. Respiration and fertilizing capacity of sea urchin sperm in presence of serum albumin and jelly coat solution, 324.
Vertebrates, pituitary effects on chromatophores of, 487.
VILLEE, CLAUDE A., VIVIEN WHITE and A. BAIRD HASTINGS. Studies on carbohydrate metabolism in mammalian muscle, 312.
VILLEE, CLAUDE A., K. LINDERSTRØM-LANG, M. OTTESEN, C. B. ANFINSEN and D. STEINBERG. The enzymatic conversion of ovalbumin to plakalbumin, 322.
VILLEE, CLAUDE A., ROBERT LICHTENSTEIN, NEAL NATHANSON and BRITA ROLANDER. Studies of the carbohydrate metabolism of invertebrate tissues in vitro, 365.
VILLEE, CLAUDE A., ROBERT LICHTENSTEIN, NEAL NATHANSON and BRITA ROLANDER. Phosphorus metabolism in embryonic and adult invertebrate tissues, 366.

VISHNIAC, WOLF, ROY KISLIUK and SEYMOUR H. HUTNER. Ionic balance required by some marine microorganisms and effect of quaternary ammonium ions, 328.
Vitamin A, action of alcohol dehydrogenase on, 330.

WALTERS, C. P. *See* G. H. A. CLOWES, 335.
WALTERS, C. P. *See* A. K. KELTCH, 334.
WALTERS, C. P. *See* PHILIPP STRITTMATTER, 333.
WALTERS, VLADIMIR. *See* P. F. SCHOLANDER, 225, 237, 259.
WARNER, ROBERT C. and IONE WEBER. Preparation and properties of the conalbumin of egg white, 323.
WARNER, ROBERT C. and MILTON LEVY. Kinetics of denaturation of several proteins, 336.
WARNER, ROBERT C. *See* MILTON LEVY, 337.
Water and biological function, 368.
Water and biological morphology, 367.
WEBER, IONE. *See* ROBERT C. WARNER, 323.
WEISEL, GEORGE F. The comparative effects of teleost and beef pituitary on chromatophores of cold-blooded vertebrates, 487.
WELLER, J. M. *See* I. M. TAYLOR, 311.
WELLS, PATRICK H. and ARTHUR C. GIESE. Photoreactivation of ultraviolet light injury in gametes of the sea urchin Strongylocentrotus purpuratus, 163.
WHITE, VIVIEN. *See* CLAUDE A. VILLEE, 312.
WICHTERMAN, RALPH. Occurrence of a new variety containing two opposite mating types of Paramecium calkinsi as found in sea water of high salinity content, 366.
WICHTERMAN, RALPH. A simple method for obtaining large numbers of fission-stages of Paramecium and certain other negatively geotropic ciliates, 366.
WICKLUND, ELSA. *See* ERIK VASSEUR, 324.
WILLIAMS, CARROLL M. and ROBERT GALAMBOS. Oscilloscopic and stroboscopic analysis of the flight sounds of Drosophila, 300.
WILSON, D. L. *See* J. F. MANERY, 312.
WILSON, WALTER L. Changes in the protoplasmic cortex during cell division, 341.
WILSON, WALTER L. *See* L. V. HEILBRUNN, 340.
WITMER, RALPH. *See* HAROLD F. BLUM, 317.
WOODWARD, A. A. Proteolytic enzymes in the eggs of the clam, Mactra solidissima, 367.
WRINCH, DOROTHY. Water and biological function, 368.
WRINCH, DOROTHY. Water and biological morphology, 367.

X-RAY disturbance of selective potassium accumulation in human erythrocytes, 310.
X-ray effects on cell division, 317.

YEAST cell, potassium and sodium content of, 319.
YUDKIN, WARREN H. Transphosphorylation in annelid muscle, 352.

ZORZOLI, ANITA. Alkaline phosphatase activity in normal and scorbutic bones, 321.

Volume 99 Number 1

99, 1950 comp
Acc. # 65213
12 22 [illegible]

THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. CONKLIN, Princeton University
DONALD P. COSTELLO, University of North Carolina
E. N. HARVEY, Princeton University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University
DOUGLAS WHITAKER, Stanford University

H. B. STEINBACH, University of Minnesota
Managing Editor

Marine Biological Laboratory
LIBRARY
SEP [illegible] - 1950
WOODS HOLE, MASS.

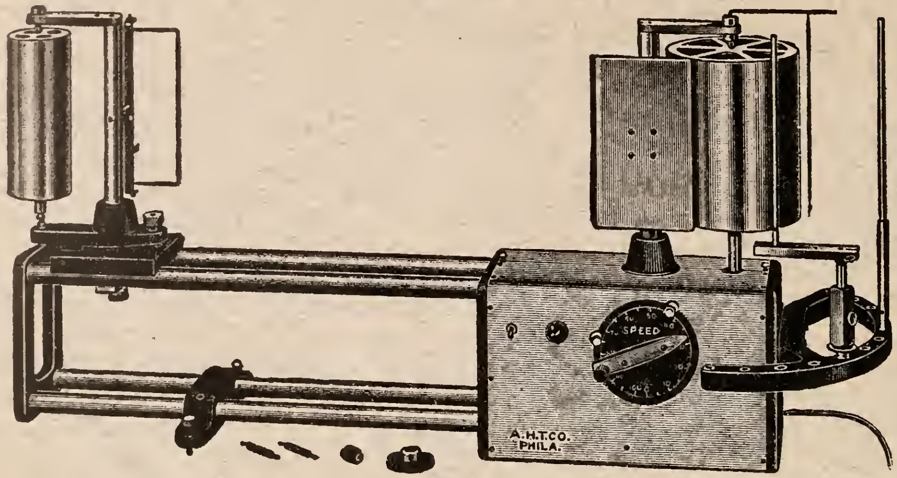
AUGUST, 1950

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

BOEHNKE

UNIVERSAL KYMOGRAPH

A new model, of great rigidity, in which instantaneous change of speed can be made simply by turning the lever on the dial



7974-P.

KYMOGRAPH, BOEHNKE UNIVERSAL. A new model, of great rigidity, driven by a 1/15 h.p. constant speed induction motor and a precision friction type transmission. Speed can be varied from 0 to 351 cm per minute and can be changed instantaneously simply by turning the lever on the dial, which indicates a direct percentage of maximum speed.

Model A, Basic Outfit. With variable speed transmission enclosed in aluminum housing, 17½ inches long × 8½ inches wide × 10¼ inches high, from which there extends a frame-type bed consisting of four lengths of anodized aluminum tubing, 1¼ inches outside diameter, and a cast aluminum endpiece. Driven drum is 8 inches diameter × 10 inches high, movable idle drum 4 inches diameter × 10 inches high. The drums are made of brass tubing with cast aluminum ends. Drum pillars are made of aluminum rod 1¼ inches diameter; shafts are Stainless steel, ¾-inch diameter, with hardened steel bearings. Writing levers, manometer, etc., can be mounted on a semicircular base which is attachable to the end of housing, if desired. Two sets of gears are supplied with each outfit.

Overall length, 48 inches.
Overall height, without table, 26½ inches.
Overall height (max.), with table, 72 inches.
Maximum paper length 112 inches.
Maximum paper width 10 inches.
Maximum paper speed (mm per minute, 3510; cm. per second, 5.85).

Maximum drum revolutions per minute, 5.5.

Minimum paper speed, 0.

Motor, induction-type, 1/50 h.p., 1725 r.p.m., 115 volts, 60 cycles, a.c. only.

Weight, without accessories, 106 lbs.

7974-P. Kymograph Outfit, Boehnke Universal, Electric, A.H.T. Co. Specification, Model A, Basic Outfit, as above described, complete with all accessories as shown in illustration and directions for use.
For use on 115 volts, 50 or 60 cycles, a.c., only **690.60**
Net weight........................130 lbs. Shipping weight....................200 lbs.

More detailed information regarding above and other models of the Boehnke Universal Kymograph, sent upon request.

ARTHUR H. THOMAS COMPANY
RETAIL—WHOLESALE—EXPORT
LABORATORY APPARATUS AND REAGENTS
WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U. S. A.
Cable Address, "BALANCE", Philadelphia

BIOLOGICAL ABSTRACTS

COVERS THE WORLD'S BIOLOGICAL LITERATURE

How do you keep abreast of the literature in your field? No individual possibly could accumulate and read all of the biological contributions in the original—yet some relatively obscure journal might publish a revealing paper on the very subject in which you are most interested.

Biological Abstracts now publishes concise, informative abridgments of all the significant contributions from more than 2,500 journals. As well as the complete edition, it also is published in nine low-priced sectional editions which are specially designed for individuals who are interested only in one or more closely related fields.

Production costs have increased to such an extent that the active support of all biologists is needed to maintain this important service. Write for full details and a sample copy of the sectional edition covering your field.

BIOLOGICAL ABSTRACTS
UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA 4, PA.

MICROFILM SERVICE

◆

The Library of The Marine Biological Laboratory can supply microfilms of material from periodicals included in its list. Requests should include the title of the paper, the author, periodical, volume and date of publication.

◆

Rates are as follows: $1.00 for papers up to 50 pages, and $.10 for each additional 10 pages or fraction thereof.

LANCASTER PRESS, Inc.

LANCASTER, PA.

* * *

THE EXPERIENCE we have gained from printing some sixty educational publications has fitted us to meet the standards of customers who demand the best.

We shall be happy to have workers at the MARINE BIOLOGICAL LABORATORY write for estimates on journals or monographs. Our prices are moderate.

INSTRUCTIONS TO AUTHORS

The Biological Bulletin accepts papers on a variety of subjects of biological interest. In general, a paper will appear within three months of the date of its acceptance. The Editorial Board requests that manuscripts conform to the requirements set below.

Manuscripts. Manuscripts should be typed in double or triple spacing on one side of paper, 8½ by 11 inches.

Tables should be typewritten on separate sheets and placed in correct sequence in the text. Explanations of figures should be typed on a separate sheet and placed at the end of the text. Footnotes, numbered consecutively, may be placed on a separate sheet at the end of the paper.

A condensed title or running page head of not more than thirty-five letters should be included.

Figures. The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. Explanatory matter should be included in legends as far as possible, not lettered on the illustrations. Figures should be prepared for reproduction as line cuts or halftones; other methods will be used only at the author's expense. Figures to be reproduced as line cuts should be drawn in black ink on white paper or blue-lined co-ordinate paper; those to be reproduced as halftones should be mounted on Bristol board and any designating letters or numbers should be made directly on the figures. The author's name should appear on the reverse side of all figures. The desired reduction should be specified on each figure.

Literature cited. The list of literature cited should conform to the style set in this issue of The Biological Bulletin. Papers referred to in the manuscript should be listed on separate pages headed "Literature Cited."

Mailing. Manuscripts should be packed flat. Large illustrations may be rolled in a mailing tube, but all illustrations larger than 9 by 12 inches must be accompanied by photographic reproductions or tracings that may be folded to page size.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost; approximate figures will be furnished upon request.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between June 15 and September 1, and to the Department of Zoology, University of Minnesota, Minneapolis, Minnesota, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

BIOLOGY MATERIALS

The Supply Department of the Marine Biological Laboratory has a complete stock of excellent plain preserved and injected materials, and would be pleased to quote prices on school needs.

PRESERVED SPECIMENS

for

Zoology, Botany, Embryology,
and Comparative Anatomy

LIVING SPECIMENS

for

Zoology and Botany

including Protozoan and Drosophila Cultures, and Animals for Experimental and Laboratory Use.

MICROSCOPE SLIDES

for

Zoology, Botany, Embryology, Histology, Bacteriology, and Parasitology.

CATALOGUES SENT ON REQUEST

Supply Department
MARINE
BIOLOGICAL LABORATORY
Woods Hole, Massachusetts

CONTENTS

Page

Annual report 1

ANDERSON, JOHN MAXWELL

A cytological and cytochemical study of the male accessory reproductive glands in the Japanese beetle, Popillia japonica Newman 49

GROSCH, DANIEL S.

Starvation studies with the parasitic wasp habrobracon 65

HAND, CADET AND JOHN R. HENDRICKSON

A two-tentacled, commensal hydroid from California (Limnomedusae, Proboscidactyla) 74

MOORE, ANNA-BETTY CLARK

The development of reciprocal androgenetic frog hybrids 88

SALVATORE, CARLOS ALBERTO

Action of estrone and progesterone on nuclear volume (studied by applying the Karyometric-statistical method) 112

SPRATT, NELSON T., JR.

Nutritional requirements of the early chick embryo. III. The metabolic basis of morphogenesis and differentiation as revealed by the use of inhibitors 120

STUNKARD, HORACE W.

Further observations on Cercaria parvicaudata Stunkard and Shaw, 1931 136

Volume 99 Number 2

THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. CONKLIN, Princeton University
DONALD P. COSTELLO, University of North Carolina
E. N. HARVEY, Princeton University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University
DOUGLAS WHITAKER, Stanford University

H. B. STEINBACH, University of Minnesota
Managing Editor

Marine Biological Laboratory
LIBRARY
DEC 7 - 1950
WOODS HOLE, MASS.

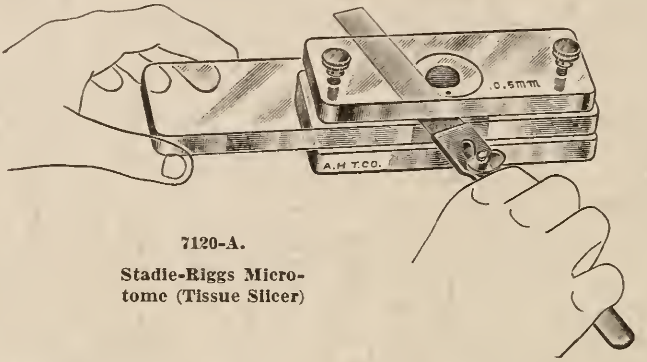
OCTOBER, 1950

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

STADIE-RIGGS IMPROVED MODEL

TISSUE SLICER

For the preparation of thin, uniform sections of fresh tissue as required for metabolic studies of surviving tissue *in vitro*



7120-A.

Stadie-Riggs Microtome (Tissue Slicer)

MICROTOME (TISSUE SLICER), Stadie-Riggs, Improved Model. For the preparation of thin, uniform sections of fresh tissue as required for metabolic studies of surviving tissue *in vitro.* See William C. Stadie and Benjamin C. Riggs, *The Journal of Biological Chemistry, Vol. 154, No. 3 (Aug., 1944), p. 669.*

Tissue is held between the recessed under surface of the cover plate and the advancing edge of a thin, sharp blade, permitting the cutting of multiple slices with considerable reproducibility and with a minimum of trauma. Sections average 0.5 mm in thickness.

The microtome consists of three plates of carefully machined and polished transparent Plexiglas, each approximately ½ inch thick, one being extended to form a handle, and a thin blade with 4-inch cutting edge and detachable handle. Top plate is 5 inches long x 1⅞ inches wide, with shallow centrally located recess approximately 32 mm diameter x 0.5 mm deep in the under surface, and with threaded holes near the ends for attachment to the middle plate. The middle plate which, in use, is fastened tightly beneath the top plate by means of two setscrews, is 8⅛ inches long, with a circular opening 32 mm diameter directly beneath the recess in the cover plate.

A slot between adjacent surfaces of the cover plate and the middle plate permits entrance of the knife blade. On the base plate is mounted a circular platform, 31 mm diameter x 12 mm high, which fits into the circular opening in the middle plate. In use, fresh tissue is placed on the platform of the base plate. The cover and middle plates are then fastened together and superimposed upon the base plate over the platform on which the tissue has been placed. Slices of tissue are cut by means of a sawing motion of the knife blade in the slot between the top and middle plates as shown in the illustration. The tissue, knife and chamber are in full view at all times. All parts are readily separated for cleaning.

7120-A. **Microtome (Tissue Slicer), Stadie-Riggs, Improved Model,** as above described, for sections 0.5 mm thick; complete with knife handle with ten blades and directions for use 28.25

7120-D. **Blades,** only, of steel, 4 inches long. Per carton of 10 .... 3.50
10% discount in lots of 5 cartons
15% " " " " 10 "

ARTHUR H. THOMAS COMPANY

RETAIL—WHOLESALE—EXPORT

LABORATORY APPARATUS AND REAGENTS

WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U. S. A.

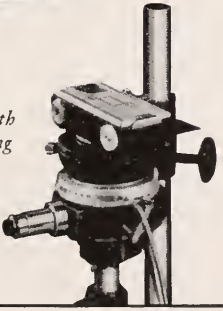
Cable Address, "BALANCE," Philadelphia

New SPENCER Photomicrographic Cameras

HERE THEY ARE!

These new 35 mm and 4″ x 5″ Photomicrographic Cameras offer many advanced and desirable features. Simple to operate, sturdily built, they are designed and priced for laboratory and clinical use. Three models are offered—4″ x 5″ Camera with Universal shutter and telescopic focusing eyepiece; 35 mm film Camera with Universal shutter and telescopic focusing eyepiece; 4″ x 5″ Camera with Alphax shutter.

- **360° REVOLVING BODY**—Orientation of 4″ x 5″ and 35 mm Camera bodies eliminates need for circular revolving stage on microscope.
- **SWING-IN LIGHT-TYPE ADAPTER**—Camera may be swung in or away from microscope without disturbing focus. Fine adjustment stop assures precise repeat positioning—faster operation.
- **TELESCOPIC FOCUSING EYEPIECE**—Permits viewing a specimen through reflex mirror up to moment shutter is released. Cross-hair reticule assists critically sharp focusing.
- **TAKES 4″ x 5″ PLATES, FILM OR FILM PACK**—Suitable adapters are available.
- **35mm CAMERA**—Interchangeable with 4″ x 5″ Camera body. Built-in compensating lens accommodates for difference in focal length.
- **FOUR POSITIONING CLAMPS**—One pair adjustable to one of three positions for convenient location of microscope.
- **RUBBER VIBRATION ABSORBERS**—Adjustable to position base firmly.
- **4″ x 5″ GROUND GLASS HAS DIAGONAL TRANSPARENT STRIPS**—Permits use of magnifier to assist in critically focusing image over entire plate.



35 mm Model with telescopic focusing eyepiece

4″ x 5″ Model with Alphax shutter

See your AO Spencer Distributor for literature describing the new AO Spencer Photomicrographic Cameras in detail, or write Dept. K185.

American Optical
COMPANY
INSTRUMENT DIVISION • BUFFALO 15, NEW YORK

INSTRUCTIONS TO AUTHORS

The Biological Bulletin accepts papers on a variety of subjects of biological interest. In general, a paper will appear within three months of the date of its acceptance. **The Editorial Board requests that manuscripts conform to the requirements set below.**

Manuscripts. Manuscripts should be typed in double or triple spacing on one side of paper, 8½ by 11 inches.

Tables should be typewritten on separate sheets and placed in correct sequence in the text. Explanations of figures should be typed on a separate sheet and placed at the end of the text. Footnotes, numbered consecutively, may be placed on a separate sheet at the end of the paper.

A condensed title or running page head of not more than thirty-five letters should be included.

Figures. The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. **Explanatory matter should be included in legends as far as possible, not lettered on the illustrations. Figures should be prepared for reproduction as line cuts or halftones; other methods will be used only at the author's expense.** Figures to be reproduced as line cuts should be drawn in black ink on white paper or blue-lined co-ordinate paper; those to be reproduced as halftones should be mounted on Bristol board and any designating letters or numbers should be made directly on the figures. The author's name should appear on the reverse side of all figures. The desired reduction should be specified on each figure.

Literature cited. **The list of literature cited should conform to the style set in this issue of The Biological Bulletin. Papers referred to in the manuscript should be listed on separate pages headed "Literature Cited."**

Mailing. Manuscripts should be packed flat. Large illustrations may be rolled in a mailing tube, but all illustrations larger than 9 by 12 inches must be accompanied by photographic reproductions or tracings that may be folded to page size.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost; approximate figures will be furnished upon request.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between June 15 and September 1, and to the Department of Zoology, University of Minnesota, Minneapolis, Minnesota, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

BIOLOGY MATERIALS

The Supply Department of the Marine Biological Laboratory has a complete stock of excellent plain preserved and injected materials, and would be pleased to quote prices on school needs.

PRESERVED SPECIMENS

for

Zoology, Botany, Embryology,
and Comparative Anatomy

LIVING SPECIMENS

for

Zoology and Botany

including Protozoan and Drosophila Cultures, and Animals for Experimental and Laboratory Use.

MICROSCOPE SLIDES

for

Zoology, Botany, Embryology, Histology, Bacteriology, and Parasitology.

CATALOGUES SENT ON REQUEST

* * *

Supply Department
MARINE
BIOLOGICAL LABORATORY
Woods Hole, Massachusetts

CONTENTS

Page

BONNER, JOHN TYLER
Observations on polarity in the slime mold Dictyostelium discoideum 143

BROWNE, MARIE J., MARJORIE W. PITTS, AND ROBERT F. PITTS
Alkaline phosphatase activity in kidneys of Glomerular and Aglomerular marine Teleosts 152

FRENKEL, ALBERT, HANS GAFFRON, AND E. BATTLEY
Photosynthesis and photoreduction in blue-green algae 157

WELLS, PATRICK H., AND ARTHUR C. GIESE
Photoreactivation of ultraviolet light injury in gametes of the sea urchin Strongylocentrotus purpuratus 163

HERLANT-MEEWIS, HENRIETTE
Cyst-formation in Aeolosoma hemprichi (Ehr) 173

MOORE, HILARY B.
The relation between the scattering layer and the Euphausiacea 181

PEISS, C. N., AND JOHN FIELD
The respiratory metabolism of excised tissues of warm- and cold-adapted fishes 213

SCHOLANDER, P. F., VLADIMIR WALTERS, RAYMOND HOCK, AND LAURENCE IRVING
Body insulation of some arctic and tropical mammals and birds 225

SCHOLANDER, P. F., RAYMOND HOCK, VLADIMIR WALTERS, FRED JOHNSON, AND LAURENCE IRVING
Heat regulation in some arctic and tropical mammals and birds 237

SCHOLANDER, P. F., RAYMOND HOCK, VLADIMIR WALTERS, AND LAURENCE IRVING
Adaptation to cold in arctic and tropical mammals and birds in relation to body temperature, insulation and basal metabolism 259

TAYLOR, WILLIAM RANDOLPH
Reproduction of Dudresnaya crassa Howe 272

TURNER, C. L.
The reproductive potential of a single clone of Pelmatohydra oligactis 285

WILLIAMS, CARROLL M., AND ROBERT GALAMBOS
Oscilloscopic and stroboscopic analysis of the flight sounds of Drosophila 300

Papers presented at the meeting of the Society of General Physiologists 308

Abstracts of seminar papers presented at the Marine Biological Laboratory 321

Reports on Lalor Fellowship Research 369

Volume 99 Number 3

THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

E. G. CONKLIN, Princeton University
DONALD P. COSTELLO, University of North Carolina
E. N. HARVEY, Princeton University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University
DOUGLAS WHITAKER, Stanford University

H. B. STEINBACH, University of Minnesota
Managing Editor

Marine Biological Laboratory
LIBRARY
JAN 17 1951
WOODS HOLE, MASS.

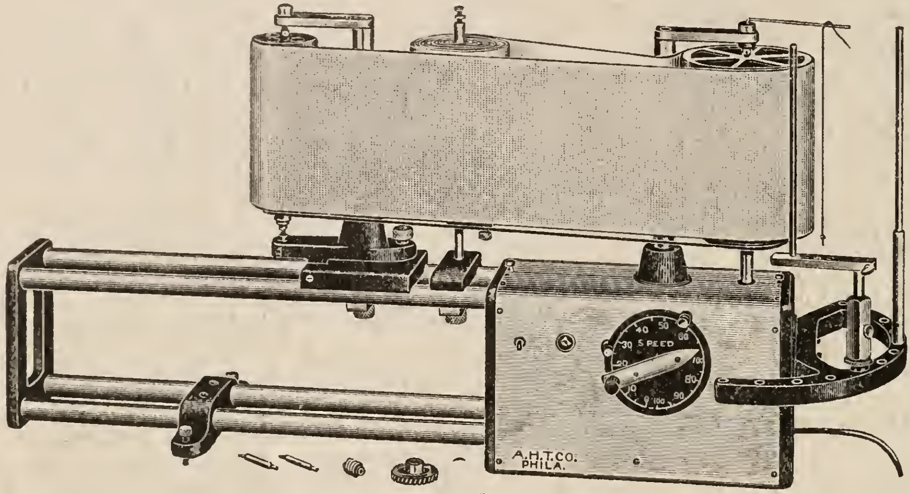
DECEMBER, 1950

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

BOEHNKE CONTINUOUS PAPER MODEL

UNIVERSAL KYMOGRAPH

A new model, of great rigidity, in which instantaneous change of speed can be made simply by turning the lever on the dial



7976-J.

KYMOGRAPH BOEHNKE UNIVERSAL, Continuous Paper Model, for making ink records. Can be adapted in the laboratory for use with smoked paper tracings. A new model, of great rigidity, driven by a 1/15 h.p. constant speed induction motor and a precision friction type transmission. Speed can be varied from 0 to 351 cm per minute and can be changed instantaneously simply by turning the lever on the dial, which indicates a direct percentage of maximum speed.

With variable speed transmission enclosed in aluminum housing, 17½ inches long × 8½ inches wide × 10½ inches high, from which there extends a frame-type bed consisting of four lengths of anodized aluminum tubing, 1¼ inches outside diameter, and a cast aluminum endpiece. Driven drum is 8 inches diameter × 10 inches high, movable idle drum 4 inches diameter × 10 inches high. The drums are made of brass tubing with cast aluminum ends. Drum pillars are made of aluminum rod 1¼ inches diameter; shafts are Stainless steel, ¾-inch diameter, with hardened steel bearings. Writing levers, manometer, etc., can be mounted on a semicircular base which is attached to the end of housing.

7976-J. Kymograph Outfit, Boehnke Universal, Continuous Paper Model, as above described, complete with accessories as shown in illustration, including two sets of driving gears, semi-circular base, Stainless steel posts and rods, leveling foot, holder for the roll of paper, friction roller to maintain proper paper tension, two pulleys and belt to synchronize turning of drums and paper roll, roll of glazed paper, aluminum platen—not shown in illustration—to provide a flat writing surface, and directions for use. For 115 volts, 50 or 60 cycles a.c. **$840.30**
Net weight .... 135 lbs. Shipping weight .... 210 lbs.

More detailed information regarding above and other models of the Boehnke Universal Kymograph, sent upon request.

ARTHUR H. THOMAS COMPANY
RETAIL—WHOLESALE—EXPORT
LABORATORY APPARATUS AND REAGENTS
WEST WASHINGTON SQUARE PHILADELPHIA 5, PA., U. S. A.

Cable Address, "BALANCE," Philadelphia

BIOLOGICAL ABSTRACTS

COVERS THE WORLD'S BIOLOGICAL LITERATURE

How do you keep abreast of the literature in your field? No individual possibly could accumulate and read all of the biological contributions in the original—yet some relatively obscure journal might publish a revealing paper on the very subject in which you are most interested.

Biological Abstracts now publishes concise, informative abridgments of all the significant contributions from more than 2,500 journals. As well as the complete edition, it also is published in nine low-priced sectional editions which are specially designed for individuals who are interested only in one or more closely related fields.

Production costs have increased to such an extent that the active support of all biologists is needed to maintain this important service. Write for full details and a sample copy of the sectional edition covering your field.

BIOLOGICAL ABSTRACTS
UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA 4, PA.

MICROFILM SERVICE

◆

The Library of The Marine Biological Laboratory can supply microfilms of material from periodicals included in its list. Requests should include the title of the paper, the author, periodical, volume and date of publication.

◆

Rates are as follows: $1.00 for papers up to 50 pages, and $.10 for each additional 10 pages or fraction thereof.

LANCASTER PRESS, Inc.

LANCASTER, PA.

☆

THE EXPERIENCE we have gained from printing some sixty educational publications has fitted us to meet the standards of customers who demand the best.

We shall be happy to have workers at the MARINE BIOLOGICAL LABORATORY write for estimates on journals or monographs. Our prices are moderate.

INSTRUCTIONS TO AUTHORS

The Biological Bulletin accepts papers on a variety of subjects of biological interest. In general, a paper will appear within three months of the date of its acceptance. **The Editorial Board requests that manuscripts conform to the requirements set below.**

Manuscripts. Manuscripts should be typed in double or triple spacing on one side of paper, 8½ by 11 inches.

Tables should be typewritten on separate sheets and placed in correct sequence in the text. Explanations of figures should be typed on a separate sheet and placed at the end of the text. Footnotes, numbered consecutively, may be placed on a separate sheet at the end of the paper.

A condensed title or running page head of not more than thirty-five letters should be included.

Figures. The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. **Explanatory matter should be included in legends as far as possible, not lettered on the illustrations. Figures should be prepared for reproduction as line cuts or halftones; other methods will be used only at the author's expense.** Figures to be reproduced as line cuts should be drawn in black ink on white paper or blue-lined coordinate paper; those to be reproduced as halftones should be mounted on Bristol board and any designating letters or numbers should be made directly on the figures. The author's name should appear on the reverse side of all figures. The desired reduction should be specified on each figure.

Literature cited. **The list of literature cited should conform to the style set in this issue of The Biological Bulletin. Papers referred to in the manuscript should be listed on separate pages headed "Literature Cited."**

Mailing. Manuscripts should be packed flat. Large illustrations may be rolled in a mailing tube, but all illustrations larger than 9 by 12 inches must be accompanied by photographic reproductions or tracings that may be folded to page size.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost; approximate figures will be furnished upon request.

THE BIOLOGICAL BULLETIN

The Biological Bulletin is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $1.75. Subscription per volume (three issues), $4.50.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Massachusetts, between June 15 and September 1, and to Dr. Donald P. Costello, Department of Zoology, University of North Carolina, Chapel Hill, North Carolina, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa.
under the Act of August 24, 1912.

BIOLOGY MATERIALS

The Supply Department of the Marine Biological Laboratory has a complete stock of excellent plain preserved and injected materials, and would be pleased to quote prices on school needs.

* * *

PRESERVED SPECIMENS

for

Zoology, Botany, Embryology, and Comparative Anatomy

LIVING SPECIMENS

for

Zoology and Botany

including Protozoan and Drosophila Cultures, and Animals for Experimental and Laboratory Use.

MICROSCOPE SLIDES

for

Zoology, Botany, Embryology, Histology, Bacteriology, and Parasitology.

CATALOGUES SENT ON REQUEST

* * *

Supply Department

MARINE
BIOLOGICAL LABORATORY
Woods Hole, Massachusetts

CONTENTS

Page

BOWDEN, BERNARD J.
Some observations on a luminescent freshwater limpet from New Zealand 373

BURNS, JEAN, AND D. EUGENE COPELAND
Chloride excretion in the head region of Fundulus heteroclitus 381

COSTELLO, DONALD P., AND CATHERINE HENLEY
Heteroploidy in Triturus torosus. II. The incidence of chromosomal variations in shipped larvae 386

DAN, JEAN C.
Sperm entrance in echinoderms, observed with the phase contrast microscope 399

DAN, JEAN C.
Fertilization in the medusan, Spirocodon saltatrix 412

FREEMAN, JOHN A.
Oxygen consumption, brain metabolism and respiratory movements of goldfish during temperature acclimatization, with special reference to lowered temperatures 416

GATES, G. E.
Regeneration in an earthworm, Eisenia foetida (Savigny) 1826. III. Regeneration from simultaneous anterior and posterior transitions 425

GETMAN, HERBERT C.
Adaptive changes in the chloride cells of Anguilla rostrata .. 439

HASSETT, C. C., V. G. DETHIER, AND J. GANS
A comparison of nutritive values and taste thresholds of carbohydrates for the blowfly 446

KLEINHOLZ, L. H., with the assistance of V. J. HAVEL AND R. REICHART
Studies in the regulation of blood-sugar concentration in crustaceans. II. Experimental hyperglycemia and the regulatory mechanisms 454

REID, W. MALCOLM
Glycogen depletion during starvation in the nemertean, Micrura leidyi (Verrill), and its ecological significance 469

TEWINKEL, LOIS E.
Notes on ovulation, ova, and early development in the smooth dogfish, Mustelus canis 474

WEISEL, GEORGE F.
The comparative effects of teleost and beef pituitary on chromatophores of cold-blooded vertebrates 487

WH 1AYZ 0